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## SOME CASES OF CUTANEOUS MYIASIS, WITH NOTES ON THE LARVAE OF *WOHLFAHRTIA* *VIGIL* (WALKER)

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In this JOURNAL, the writer published (1920) a report of two cases of cutaneous myiasis in very young infants, both of which occurred at Toronto, Ont., during June, 1919. From the second case four adults of *Wohlfahrtia vigil* (Walker) were reared and from a comparison of the larvae taken from the two cases and the close similarity of the clinical pictures which they presented, it was decided beyond any doubt that the first case was due to the same species of fly.

These two cases were of special interest, owing to the fact that nothing had been previously known of the habits of either of the two North American species of *Wohlfahrtia*, although a European species, *W. magnifica*, is well known as a parasite of both man and certain of the domestic animals, having habits similar to those of the American Screw-worm Fly (*Chrysomyia macellaria*). A difference, however, was noted in the cases described and those due to *W. magnifica*, as described by Portchinsky and others, in so far as, in the latter, the larvae are always described as entering through one of the natural orifices, such as the nose, mouth or ear, or through a wound or sore, while in the cases due to *W. vigil* the lesions were scattered over an otherwise healthy and uninjured skin.

During 1921 another case occurred at Toronto and the writer was again successful in rearing *W. vigil* from it. This case was in a boy 5 months old (Pl. XXX) \* and was brought to the Sick Children's Hospital from the Infants' Home on September 2. Most of the lesions were clustered together on the left side of the neck under the angle of the jaw, one being on the left cheek. They had been first noticed by the mother 24 hours earlier, and when seen by the writer they were already secondarily infected with pus organisms, the child being in a poor general condition and suffering from an intestinal disorder. They were similar to the lesions observed in the previous cases, each being a boil-

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\* I am indebted to Dr. M. J. Brady for the excellent photograph of this case.

like sore with an external opening, and from these openings five or six larvae had already been expressed. Only three additional larvae were obtained, these measuring 5 to 7 mm. in length. Each was placed on raw beef in a separate test-tube, plugged with cotton wool. In 24 hours they reached a length of 12 to 13 mm. and in another 24 hours they were full-grown, each measuring about 17 mm. in length.

On the third day after their removal from the child the larvae were placed with the meat in a jar of earth and immediately burrowed into the latter. Next day they were at the bottom of the jar and two of them had begun to contract. Three days later they were dug up and all had transformed into puparia.

On September 27, 18 days later, a male *Wohlfahrtia virgil* emerged. I waited for the others to appear until October 5, but neither having emerged by that time I opened one of the puparia on that day and the other a week later, and in both I found pupae which had evidently died some time before the proper time for emergence, as they were quite colorless. In this case, like the previous ones, the child recovered rapidly after the removal of the maggots.

Since publishing my account of the first two cases I cleared several of the larvae with potash, including the only larva I have from case 2. Two of the larvae from case 1 belong to the first instar, the others to the second, while the single specimen from case 2 belongs to the third instar.

The first instar has recently been described by Johannsen (1921), who also determined that *W. virgil* is larviparous like other Sarcophagidae. No description has yet appeared, however, of the bucco-pharyngeal sclerites of the first and second instars, and it seems desirable that these should be described, in order to facilitate the recognition of this parasite in any stage in which it may be found. In this connection I wish to thank Professor Johannsen for his kindness in sending me one of the three larvae on which his description was based.

In Professor Johannsen's specimen of the first instar (Figs. 1, 4) the cuticular spines are somewhat larger than in the second stage, but this is scarcely noticeable in my specimens, which are a little more advanced in development and were apparently boiled in too strong a solution of potash. The posterior spiracles have two slits each and the anterior spiracular processes, though not yet apparent externally, can be seen beneath the skin. They are very small, but the ten spiracular papillae are already visible.

In the second instar the cuticular spinules are still fairly prominent, the posterior spiracles are relatively larger, with two slits each, and the anterior spiracular processes are already well developed, each bearing 9 or 10 spiracular papillae.



In the third instar the spinules are very minute and unchitinized. The anterior spiracular processes (Fig. 6) are practically unchanged. The posterior spiracles (Fig. 7) have three nearly vertical slits, somewhat convergent ventrad, the outer slit being a trifle shorter than the other two. Each is divided very unequally into a series of openings, some rounded, others subrectangular, by transverse bars of very irregular form. A submarginal series of short, spine-like cuticular processes project inward toward the orifices.

#### *The Bucco-Pharyngeal Sclerites*

*First Instar* (Figs. 1, 4, 5).—In his description of this stage Johannsen states that there is a pair of lateral mouth-hooks but no median hook, such as is described by Portchinsky (1875) as occurring in the first stage of *W. magnifica*. The median hook, which according to Keilin (1915) occurs in the primary larvae of nearly all Cycloraphous Diptera, must have been completely retracted in the specimens examined by Johannsen, for it is much more prominent than the lateral ones, which are, in fact, not the true lateral hooks at all, but the oral rods (*baguettes orales* of Keilin), the true paired hooks or "mandibular sclerites" first appearing in the second instar.

The median or "labral" hook (*mh*) arises from a slightly divided base, immediately in front of the pharyngeal sclerites and is strongly decurved, the pointed apex projecting slightly from the front part of the oral aperture in the usual position. The lateral, oral rods (*or*) are well developed, though not very strongly chitinized. Each is divided into a short, subvertical, proximal piece, situated laterad of the basal half of the median hook, and a larger and more slender, distal, horizontal piece, bounding the oral aperture laterally, and terminating just in front of the latter in a slightly projecting apex, which is indistinctly toothed.

The pharyngeal or lateral sclerites (*ps*) are narrow in the middle; the dorsal arch is narrow and extends well forward, while the dorsal and ventral cornua are long and slender, the former being the larger and more heavily chitinized (Fig. 1).

*Second Instar* (Fig. 2).—At this stage the median hook has of course disappeared, being replaced by the lateral mouth-hooks (*lh*). These have a stout base, somewhat elevated above, and produced ventrad into a stout process. From this base they are regularly curved and tapered, terminating in slender apices. Close to the ventral process is a slight chitinization, which is apparently the rudiment of the dentate sclerite (*ds*). The hypostomal sclerite (*hs*), or "*pièce intermédiaire*," is fully separate from the pharyngeal sclerites and is of large size, with a prominent ventral projection.

The pharyngeal sclerites have changed considerably in form. The lateral wings are broader in the middle. The dorsal cornua are deeply bifurcate, the lower branch being the longer, the slenderer and the more strongly chitinized. The ventral cornua are also bifurcated, but the branches are as yet ill-defined.

*Third Instar* (Fig. 3).—The bucco-pharyngeal apparatus of this stage has already been described from the structure removed from the puparium, but it was discovered afterwards that the tips of the mouth-hooks were broken off. They have in the specimen examined a relatively smaller base, which is not elevated dorsally, but is more angular ventrally than in the second instar; and the hooks are a little stouter and are strongly decurved. A small dentate sclerite is present, and is separate from its fellow of the opposite side. The hypostomal sclerite is relatively smaller. The dorsal arch of the pharyngeal sclerites projects farther forward; the bifurcations of the dorsal cornua are broader and more strongly defined, as are also the ventral cornua, the bifurcations of the latter being much shorter than those of the former.

A fourth case of cutaneous myiasis, probably due to *Wohlfahrtia vigil*, was kindly reported to me by Dr. W. D. Wiley of Brantford, Ont., where the case was observed during June, 1921. The infant was three weeks old and had been placed outside in the shade of a tree by its mother. The lesions, from which the maggots were removed, consisted of 12 or 14 "small, slightly red, pustular spots" distributed "on the front and right side of the neck, and on the anterior surface of the right forearm, or, as the mother remarked 'just on the exposed parts.'"

Finally, a case may be mentioned that occurred at Pittsburgh, Pa., in October, 1920, of an infant that was severely infested with fly larvae, but of a different species. Two specimens of the larvae from this case were received by Dr. L. O. Howard, Chief of the U. S. Bureau of Entomology, who referred them to Dr. J. M. Aldrich. To Dr. Howard I am indebted for correspondence containing data on the clinical features of the case, and to Dr. Aldrich I owe the privilege of studying the larvae. The physician in charge of the case states that the child's body was "alive with these crawlers and was covered with a miliaria and a few furuncles. The mother noticed some of the parasites emerging from the furuncles and from the rectum and vagina."

The two larvae measured 2.4 and 2.5 mm. in length and both belong to the first instar (Fig. 8). They differ very considerably from *Wohlfahrtia*, being, in fact, not Sarcophagids, but resembling much more closely the primary larvae of *Musca* and *Muscina*. In fact they resemble the first instar of *Musca domestica* very closely, except in the points given below.



WALKER—CUTANEOUS MYIASIS



PLATE I

Male infant, five months old, infected with larvae of *Wohlfahrtia vigil* (Walk.). Case 3.

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EXPLANATION OF PLATES

EXPLANATION OF PLATE II

*Wohlfahrtia vigil* (Walk.); larval structures.

Fig. 1.—First instar, lateral view of anterior segments; *an*, antenna; *mp*, so-called maxillary palpus; *mh*, median hook; *or*, oral rods; *ps*, pharyngeal sclerite; *da*, dorsal arch; *dc*, dorsal cornua; *vc*, ventral cornua.

Fig. 2.—Second instar, lateral view of bucco-pharyngeal sclerites; *lh*, lateral mouth-hooks; *ds*, dentate sclerite; *hs*, hypostomal sclerite; other lettering as before.

Fig. 3.—Third instar, lateral view of bucco-pharyngeal sclerites; *asp*, anterior spiracular process; other lettering as before.

Fig. 4.—First instar, ventral view of anterior end of body; from Professor Johannsen's specimen. Lettering as before.

Fig. 5.—First instar; ventral view of bucco-pharyngeal sclerites; larva from Case 1.

Fig. 6.—Third instar; left anterior spiracular process.

Fig. 7.—Third instar; left posterior spiracular plate.

WALKER—CUTANEOUS MYIASIS

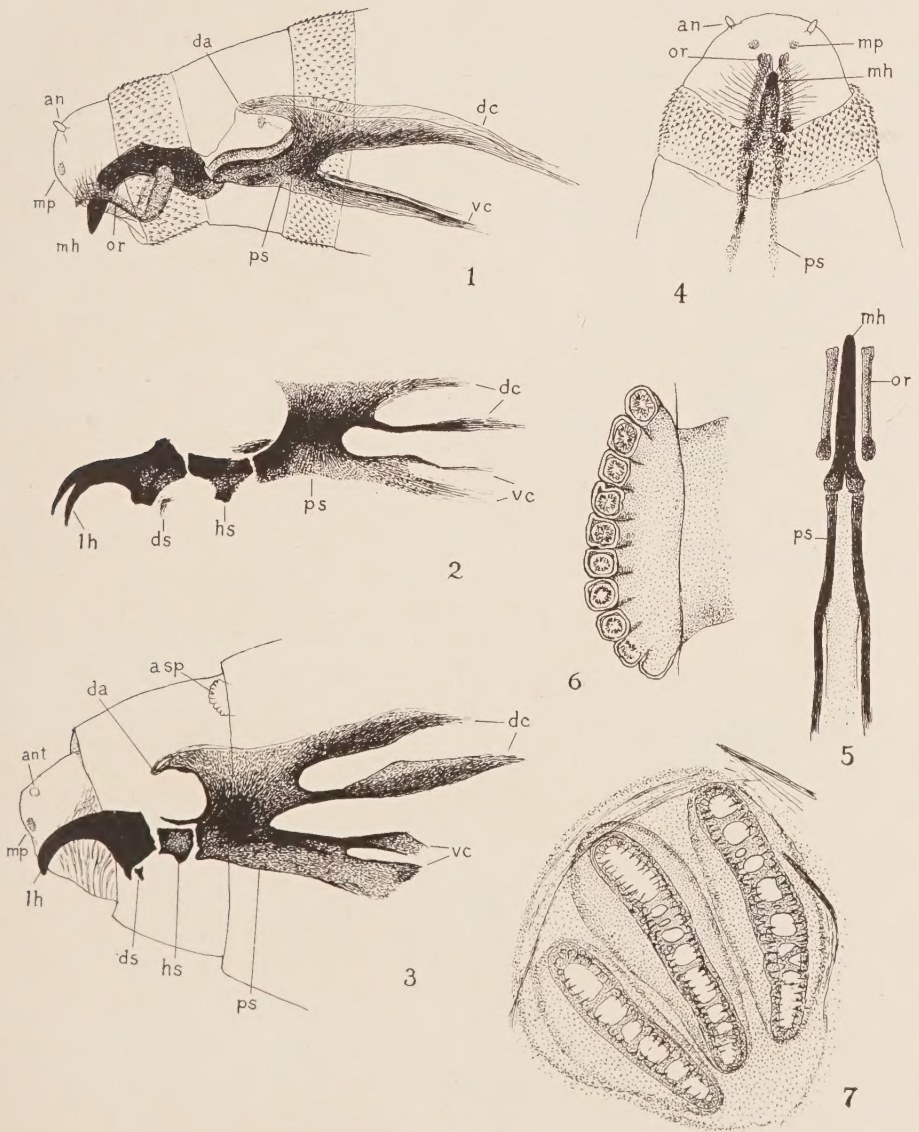
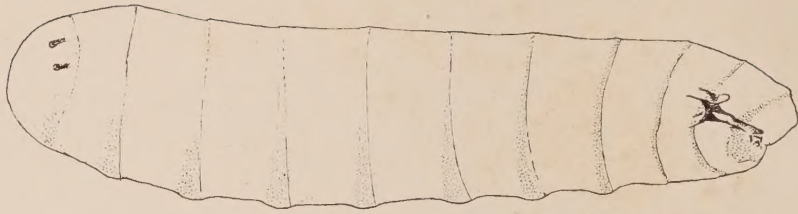
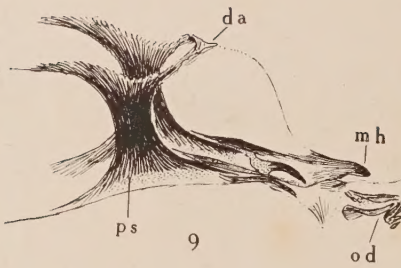


PLATE II





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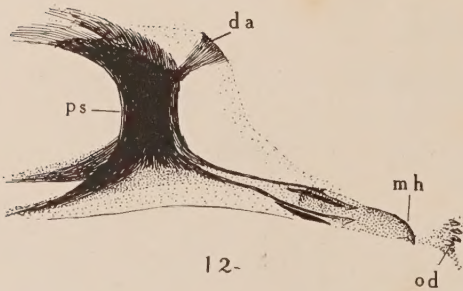
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PLATE III

Fig. 8.—Larva from the Pittsburgh case.

Fig. 9.—Same; bucco-pharyngeal sclerites; *od*, denticles around the mouth; other lettering as before.

Fig. 10.—Same; spinules from the ventral portions of the first two annuli.

Fig. 11.—Same; posterior spiracular plates.

Fig. 12.—*Musca domestica* L.; bucco-pharyngeal sclerites.



In the house-fly there are no complete spinulose annuli, while in the Pittsburgh larvae segments 2 to 5 each have a narrow annulus, which is complete and scarcely widened ventrally, except slightly on segment 5. On segment 6 the annulus is widened below but narrows above almost to the vanishing point, while on the remaining segments the annuli are incomplete dorsally, becoming successively more restricted to the ventral surface. The pharyngeal sclerites (Fig. 9) are similar to those of *Musca* (Fig. 12) but the dorsal arch is much narrower, and the anterior, ventral prolongations, from which are developed the hypostomal sclerites, are considerably shorter. The median hook is of similar size but is somewhat less abruptly pointed at the apex. The denticles around the oral aperture (*od*) are coarser than in *Musca*. The posterior end of the body is convexly rounded, without papillae, and the posterior spiracles are not sunk into a pit as they are in the Sarcophagidae. Each has two slits, but the outer margins of these slits are continuous ventrad with one another (Fig. 11). Each is bordered with small, inwardly directed, chitinous processes.

I have as yet been unable to identify the species to which these larvae belong, but it is hoped that the descriptive notes and figures here given will make possible their determination at some future time when the earliest stages of our muscoid larvae are better known.

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## CORRECTION

In my paper cited above (Walker, 1920: 3, last paragraph), I carelessly ascribed the genus *Wohlfahrtia* to Aldrich, whereas it was erected by Brauer and von Bergenstamm (Zweifel. Kais. Mus., 4: 123, 1889).

### THREE NEW SPECIES OF HOLOSTOMIDAE

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Very few studies have been made on holostomes from North America. Only a few scattered records, principally of European species, have been reported from this continent. Practically no work has been done on the endemic species. A survey of the records of species known to exist here, either in larval form or as adults, shows the limitations of this work.

Leidy (1856) and Stiles and Hassall (1894:248) recorded the occurrence of *Strigea* (*Holostomum*) *cornu* Nitzsch, a European species from the intestine of *Ardea herodias*. At the same time Leidy described another form, *Holostomum nitidum*, from the intestine of *Rana pipiens*, which Stafford (1902:724) maintains is a distome and should not be classed in this group. In 1858, Leidy reported *Diplostomulum grande* Diesing from *Strix nivea* and also noted later (1890:416) *Tetracotyle typica* Diesing from snails, *Lymnaea catascopium* and *Physa heterostropha*. Rettger (1897:224) mentions a larval holostome with some notes on its life history but does not describe or name it. *Diplostomulum parvulum* has been described by Stafford (1904:494) as a new species from fishes of Canada. Cooper (1915:191) reported finding *Diplostomulum cuticola* (v. Nordm.) encysted in the mesenteries, livers and kidneys of several Canadian fishes and *D. volvens* v. Nordm in the lens of the eye of *Micropterus dolomieu*. *Hemistomum craterum* was described from the muskrat by Barker and Noll (1915:191), and according to Stiles and Hassall (1894:248), an unnamed species of this genus was recorded from *Didelphis virginiana* by C. Curtice. *Polycotyle ornata* Willemoes-Suhm was taken from *Alligator lucius* (cited after Ward 1918:410). Faust (1917:62) in his life history studies has thrown much light on the development of holostomes. In this work he described three larval forms, *Cercaria ptychocheilus*, *Cercaria* (*Tetracotyle*) *flabelliformis*, from snails and *Tetracotyle pipientis* from *Rana pipiens*.

During the autumn of 1919, several loons (*Gavia immer*) were seen in the vicinity of Stillwater, Oklahoma, which is an unusual occurrence. In October of that year three loons were killed and brought to the laboratory. These birds harbored two species of holostomes, one belonging to the genus *Strigea* Abildgaard 1790, and the other to *Hemistomum* Diesing 1850. These forms proved to be new species and

were reported in a preliminary note (Guberlet, 1922). On Nov. 3, 1921, a ring-billed gull (*Larus delawarensis*) was killed in the same locality. This bird also yielded two species of holostomes. One was *Strigea aquavis*, the same species as taken from the loon, and the other a new species of Hemistomum.

*Strigea aquavis* nov. spec. (Figs. 1-3)

A superficial study of living specimens showed them to be sluggish. The movements of the body were very slow and apparently the worms can make but little progress in moving about on a solid sub-stratum. The principal movements displayed were slow contractions and expansions. The lamellae of the adhesive organ move about somewhat, apparently acting to some extent as feelers. They may spread out and cover an area considerably larger than the mouth of the cup-like cephalic region.

The bulbular cephalic region is opaque in the living specimen. The cylindrical caudal region is opaque when viewed from dorsal or ventral surfaces. In lateral view the upper half is transparent, while the lower half, containing the alimentary tract, vitellaria, and other organs, is opaque. The testes and ovary are also more or less colorless but their outlines can be distinctly made out. Laurer's canal may be seen coming from the oviduct near the ovary and extending to the dorsal surface. Convolutions of the uterus may be observed in the region anterior to the ovary. The eggs are yellowish brown in color.

Specimens of this trematode range in length from 2.5 to 3.5 mm. A definite constriction marks the division between the cephalic and caudal regions. The cup-shaped cephalic region, 0.5 to 0.75 mm. in length and 0.6 to 0.9 mm. in diameter, contains the oral sucker, acetabulum, and leaf-like adhesive organ. Within the caudal region, 2 to 3 mm. in length and 0.45 to 0.55 mm. in diameter, are located the reproductive organs, the excretory system and the intestinal crura.

The oral sucker, 110 to 137 $\mu$  in diameter, is terminal, being located inside of the cephalic region. The acetabulum, 143 to 192 $\mu$  in length and 126 to 154 $\mu$  in diameter, is located posterior to the oral sucker inside of the cephalic region. Attached to the bottom, inside of the cup-like cephalic region, is the hold-fast organ, or adhesive disc, lying directly ventral to the acetabulum. The lamellae, or leaf-like papillae, that form the disc extend forward and protrude beyond the rim of the cup. These lamellae are cleft for some distance, which may give the appearance of the presence of several structures, but as a rule the protuberances are so closely associated that the divisions are not apparent. Slightly posterior to the base of the disc is the adhesive gland, 134 $\mu$  in length and 210 $\mu$  in width.



The mouth is located in the oral sucker. Directly posterior to it is the small pharynx, 70 to 88 $\mu$  in length and 55 to 70 $\mu$  in diameter. There is practically no esophagus as the alimentary canal bifurcates almost immediately forming the intestinal crura. These pass on either side of the acetabulum and downward to the ventral region of the body where they lie between the vitellaria and extend to the posterior end of the body.

The excretory system in this species is difficult to make out on account of the exceedingly large and numerous excretory spaces that fill in the areas around the various organs. In the living specimen certain parts could be traced only with difficulty because of the opacity of the lower half of the worm. The larger canals could be made out to some extent by detecting movements of small semi-transparent granules flowing in the liquid contents. Two principal lateral canals form the basis for the system and these are united by a network of smaller canals. The lateral canals unite in the posterior end of the body and empty at the common excretory pore. A complete study of the excretory system could not be made on account of a lack of suitable material.

*Reproduction Organs.*—Two large testes, one in front of the other, are located directly posterior to the ovary. These are irregular in shape and composed of four lobes each; they measure 0.37 to 0.44 mm. in length, 0.296 to 0.44 mm. in width, and 0.30 to 0.33 mm. in thickness. The anterior testis is slightly smaller than the measurements given. Vasa efferentia arise from the anterior edge of each testis. The duct from the anterior testis forms a seminal reservoir ventral to the ovary. From this reservoir the duct passes posteriad, and unites with the one from the posterior testis, forming the vas deferens which passes posteriorly to the vesicula seminalis. The vesicula seminalis empties into an ejaculatory pouch which has some of the characteristics of a cirrus pouch. This structure is 0.162 to 0.185 mm. in length and is 0.088 mm. in diameter. It has a heavy wall but it is not muscular. There is not a true cirrus. This modification of the vesicula seminalis approaches somewhat the structure of a cirrus pouch. Since this is not a true cirrus pouch it agrees with the characteristics of the genital organs of the Holostomidae, as given by Brandes (1888: 426, 1890: 579) and Lühe (1909: 160). Odhner (1913: 308) and Faust (1921: 82), however, both show the presence of this organ in *Cyathocotyle Mühlingi*.

In *Strigea aquavivis* there is a modified bursa surrounding the genital cone. During certain movements of the living specimen the cone may be extruded from the pit.

The ovary is dorsal and lies near the middle of the caudal region

of the body. It is somewhat irregular in shape varying in length from 0.14 to 0.25 mm., in breadth 0.2 to 0.247 mm., and in thickness 0.177 to 0.2 mm. The oviduct arises at the posterior dorsal edge of the ovary and passes posteriad for some distance, giving off Laurer's canal almost immediately after leaving the ovary. Laurer's canal extends dorsad to the dorsal surface of the body. The oviduct continues posteriorly and somewhat ventrad to Mehlis' gland, a group of unicellular gland cells which are somewhat scattered and do not form a compact mass. As the oviduct passes through Mehlis' gland it becomes the ootype and at this point receives the vitelline canal. The oviduct upon leaving Mehlis' gland enlarges into the uterus which turns downward between the testes, becomes convoluted and passes anteriad. In the region ventral and slightly anterior to the ovary the uterus becomes thick walled for some distance. The uterus, somewhat convoluted, extends anteriorly almost to the anterior end of the caudal region where it bends back on itself and passes posteriad almost in a direct line to the posterior end of the body where it opens to the exterior through the genital pore in the genital cone. A small sphincter muscle surrounds the vagina in the region of the genital pore.

The vitellaria form a dense layer in the ventral half of the caudal region of the body. Small vitelline canals arise and pass dorsad to form the vitelline reservoir between the two testes. The vitelline duct passes dorsad from the reservoir and empties into the oviduct in the region of Mehlis' gland.

The eggs of *Strigca aquavis* range in length from 86 to 99 $\mu$  by 56 to 71 $\mu$  in width. An average measurement taken of a large number of eggs gave 92 by 63 $\mu$ .

Hosts: *Gavia immer* and *Larus delawarensis*.

*Hcmistomum gavium* nov. spec. (Figs. 10-13)

These are small trematodes 1 to 1.5 mm. in length. The body is in two regions of which the cephalic is much elongated and spoon-shaped, while the caudal is cylindrical. The cephalic region, comprising two-thirds to three-fifths of the length of the body, is 0.30 to 0.40 mm. in breadth and 0.094 mm. in thickness. In this region are located the oral sucker, ventral sucker, adhesive organ, and a pair of suckorial organs. The caudal region, 0.24 to 0.28 mm. in diameter, contains the reproductive organs.

The oral sucker 60 $\mu$  in length and 80 $\mu$  in diameter, is terminal and slightly ventral. Near the center of the cephalic region is located the acetabulum 70 $\mu$  in diameter. The adhesive disc is an oblong, somewhat of a two-lipped structure and more or less muscular. It has a length of 135 to 175 $\mu$  and is 0.1 mm. in breadth. This disc is on the ventral

surface of the cephalic region and located posterior to the acetabulum but does not cover it. Immediately dorsal and posterior to the adhesive disc is the adhesive gland. The lateral suctorial organs are located on either side of the oral sucker. They are apparently adhesive in function and appear somewhat glandular in structure.

The mouth is situated in the oral sucker and immediately posterior to it is the muscular pharynx, 50 to 70 $\mu$  in length and 37 $\mu$  in diameter. A very short esophagus bifurcates into the intestinal crura which pass to the posterior end of the body, where they terminate beneath the genital depression.

The excretory system terminates in the excretory pore, posterior to the genital pore. There is a slight enlargement where the lateral canals unite to form the common canal. As the canals extend forward they anastomose to form a loose excretory network which is present in nearly all parts of the body. No opportunity was afforded for this study on living material.

The two testes occurring in a series with the ovary are bi-lobed or somewhat dumbbell-shaped. They measure from 0.06 to 0.10 mm. in length, 0.18 to 0.25 mm. in breadth, and 0.13 to 0.15 mm. in thickness. The vas deferens arises at the right cephalic edge of the anterior testis. This duct then passes to the median line, ventral to the testes, and continues posteriad to the vesicula seminalis. A short duct also passes from the posterior testis to the vesicula seminalis. The vesicula seminalis is a large irregular shaped structure, 0.10 to 0.135 mm. in length and the same in width. An ejaculatory duct connects it with the genital pore.

The ovary is located slightly to the left of the median line in the anterior end of the caudal region of the body. This organ has a length of 0.07 to 0.094 mm., a breadth of 0.056 to 0.1 mm., and a thickness of 0.07 to 0.08 mm. The oviduct arises at the medio-posterior edge of the ovary and passes latero-posteriad for a short distance. Laurer's canal is given off from the oviduct and passes almost directly to the dorsal surface, lateral to the ovary. The oviduct continues latero-posteriorly around the anterior testis to Mehlis' gland near the lateral margin, where it forms the ootype. In passing from Mehlis' gland the oviduct becomes the uterus and turns transversely across the body to the median line, bends anteriorly and continues, ventral to the testis, forward to a point just anterior to the ovary. Here it turns ventrad and bends back upon itself and passes in the median line to the posterior part of the body where it opens to exterior through the genital pore. The genital pore is dorsal in a genital depression. The point of exit of the genital pore is located on a slight elevation within the depression.

The vitellaria are numerous and are distributed in small groups. In the caudal region they are ventral and extend from the posterior end forward around testes and ovary. In the cephalic region they are



generally distributed around the adhesive disc and acetabulum and well up toward the anterior end. The vitelline duct arises ventral to the testes and passes upward and enlarges into the vitelline reservoir which is located between the testes. A small duct extends upward from the reservoir and empties into the oviduct in the region of the ootype and Mehlis' gland.

The eggs are thin shelled and few in number, usually not more than a half dozen. They average in size  $85\mu$  in length by  $50\mu$  in breadth.

Host: *Gavia immer*.

*Hemistomum confusum* nov. spec. (Figs. 4-9)

These are small trematodes 1 to 2 mm. in length, with the caudal region of the body slightly longer than the cephalic. The flattened cephalic region measures 0.82 to 0.88 mm. in length, 0.27 to 0.33 mm. in breadth, and 0.08 mm. in thickness. The cylindrical caudal region is 0.88 to 1 mm. in length with a diameter of 0.25 to 0.33 mm.

The oral sucker is terminal and slightly ventral. It is slightly oval in shape with a length of 55 to  $70\mu$  and a breadth of 49 to  $55\mu$ . The acetabulum 60 to  $80\mu$  in diameter is situated in the middle of the ventral surface of the cephalic region. Posterior to the acetabulum is the sucking disc 110 to  $190\mu$  in length and 120 to  $165\mu$  in breadth. This disc is a double organ, or composed of two lateral lips. Above the disc is situated the adhesive gland, which is a double structure  $130\mu$  in length and  $60\mu$  in breadth. The lateral suctorial cups or organs 58 to  $70\mu$  in diameter are located on either side of the oral sucker. They are adhesive in function.

The mouth is located in the oral sucker and immediately posterior to it is the muscular pharynx, 50 to  $60\mu$  in length and 30 to  $50\mu$  in diameter. There is a very short esophagus, as the alimentary canal bifurcates almost immediately after leaving the pharynx into the intestinal crura, which pass to the posterior end of the body.

The excretory system is the same as in *H. gavium*.

The two testes, unequal in size, are slightly bi-lobed. The anterior gland measures 0.17 to 0.22 mm. in length, 0.22 to 0.26 mm. in breadth, and about 0.19 mm. in thickness, while the posterior is 0.22 to 0.26 mm. in length, 0.24 to 0.28 mm. in breadth, and 0.22 to 0.24 mm. in thickness. The vas efferens, arising at the posterior ventral edge of the anterior testis, passes forward around the gland and forms a reservoir between the testis and ovary. From this enlargement the duct passes posteriad beneath this testis and between the lobes of the posterior testis to the large vesicula seminalis. A short duct from the posterior

testis empties immediately into the vesicula seminalis. The vesicula seminalis is coiled and is connected with the genital pore by an ejaculatory duct.

The ovary is located slightly to the left of the median line about one-third of the distance from the anterior end of the caudal region. It has a length of 0.08 to 0.1 mm., a breadth of 0.9 to 0.1 mm., and a thickness of 0.09 to 0.12 mm. The oviduct arises at the medio-posterior edge of the ovary and passes latero-posteriad for some distance. Laurer's canal is given off and passes more or less directly to the dorsal surface, lateral to the ovary. A small seminal receptacle is connected with the oviduct, near the point of origin of Laurer's canal. The oviduct passes posteriad around the dorsal edge of the anterior testis to Mehlis' gland and the ootype. Mehlis' gland is a group of cells held together by a network of connective tissue cells. This mass of cells is more or less compact and forms a framework around the ootype. As the oviduct emerges from the ootype it becomes the uterus and turns laterad and cephalad, extending past the ovary for some distance, where it bends back on itself and passes almost in a direct line to the posterior end of the body where it terminates in the genital pore.

The vitellaria are numerous. They have a more definite structure in this species than in *H. garvium*. The outlines of the glands are distinct. They are generally distributed throughout the caudal region, especially posterior to the testes and anterior to the ovary. The latter area being almost completely filled with the glands. In the cephalic region the vitellaria are generally distributed posterior to the adhesive disc and gradually become fewer in number and disappear before they reach the ventral sucker. The vitelline material is collected into the vitelline reservoir which is located between the testes. A vitelline duct passes from the reservoir and empties into the oviduct in the region of the ootype.

Eggs are thin shelled and are few in number, usually not more than a dozen. They range in length from 99 to 110 $\mu$  and 60 to 66 $\mu$  in diameter.

Host: *Larus delawarensis*.

#### DISCUSSION

*Hemistomum confusum* apparently bears some resemblance to the European species *H. podomorphum* (Nitzsch). It was impossible to secure certain literature on the latter species and a comparison of the brief accounts by Diesing (1850:311) and Lühe (1909:160) throws very little light on the matter. In their accounts the principal characteristic of the species is the position and angle of attachment of the cephalic and caudal regions. Among the specimens of *H. confusum*

only an occasional, more or less distorted, individual bears any resemblance to a "human foot" (Fig. 6). The descriptions of *H. podomorphum* are inadequate, but on the whole, the differences in structure between the two forms appear great enough to warrant designating the American form as a new species.

*Strigea aquavis* differs from the other species of this genus in size, shape of the body, the relative sizes of the suckers, and the distribution of the vitellaria.

*Hemistomum gavium* differs from other species in the relative sizes of suckers and the distribution of the vitellaria.

The life histories of these forms are entirely unknown.

#### SUMMARY

*Strigea aquavis* n. sp. is described from the loon (*Gavia immer*) and the ring-billed gull (*Larus delawarensis*).

*Hemistomum gavium* from the loon and *H. confusum* from the ring-billed gull are described as new species.

These forms were taken in Oklahoma from migratory birds.

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## EXPLANATION OF PLATES

## Abbreviations

AD, Adhesive disc	OS, Oral sucker
AG, Adhesive gland	P, Pharynx
E, Egg	SD, Suctorial disc
ED, Ejaculatory duct	SR, Seminal receptacle
EJP, Ejaculatory pouch	S, Seminal reservoir
EX, Excretory pore	T, Testes
EXC, Excretory canal	U, Uterus
GC, Genital cone	V, Vitellaria
GP, Genital pore	VA, Vagina
I, Intestinal crura	VD, Vas deferens
L, Laurer's canal	VES, Vesicula seminalis
LS, Lateral suctorial cup	VR, Vitelline reservoir
O, Ovary	VS, Ventral sucker
OO, Ootype and Mehlis gland	

## EXPLANATION OF PLATE IV

Figs. 1 and 2.—Reconstruction of *Strigea aquavis*.  $\times 45$ ; 1, side view; 2, dorsal view.

Fig. 3.—*Strigea aquavis*, toto mount, dorsal view.  $\times 45$ .

Figs. 4 and 5.—*Hemistomum confusum*, toto mount.  $\times 43$ ; 4, dorsal view; 5, ventral view.

Fig. 6.—Partial reconstruction of *H. confusum*, side view.  $\times 43$ .

Fig. 7.—Section through adhesive disc, *H. confusum*.  $\times 160$ .

GUBERLET—NEW HOLOSTOMIDAE



PLATE IV

EXPLANATION OF PLATE V

Figs. 8 and 9.—Reconstruction of *H. confusum*.  $\times 120$ ; 8, dorsal view; 9, side view.

Figs. 10 and 11.—*H. gavium*, whole mount.  $\times 92$ ; 10, side view; 11, dorsal view.

Figs. 12 and 13.—*H. gavium*,  $\times 92$ ; 12, reconstruction from side view; 13, dorsal view.



GUBERLET—NEW HOLOSTOMIDAE



PLATE V



# THE DIAGNOSIS OF INTESTINAL FLAGELLATES BY CULTURE METHODS

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The study of the feces of thousands of persons during the past few years has indicated infection with *Chilomastix mesnili* in about 4 per cent. of cases and with *Trichomonas hominis* in about 3 per cent. (Hegner and Payne, 1921). These results are based on specimens obtained from patients suffering from intestinal disorders, or from other diseases not involving the alimentary canal, as well as from apparently healthy persons. The methods used for determining the presence or absence of these and other flagellates have been of various sorts and of different degrees of accuracy. No one, however, seems to have used the culture method described in this paper for purposes of diagnosis.

The intestinal flagellates of man in the probable order of their importance are as follows: (1) *Giardia lamblia* Stiles 1915, (2) *Chilomastix mesnili* (Wenyon) Alexeieff 1912, (3) *Trichomonas hominis* Davaine 1860, (4) *Embadomonas intestinalis* Wenyon and O'Connor 1917, and (5) *Enteromonas hominis* da Fonseca 1915. No species of the genus *Giardia* has ever been cultivated in artificial media and hence the culture method is not now applicable to *G. lamblia*. *Chilomastix mesnili* and *Embadomonas intestinalis* were both cultivated for the first time in this laboratory by Boeck (1921) and Hogue (1921 a), respectively, and *Trichomonas hominis* has been grown in artificial media by a number of investigators (see Hogue, 1921 b). We have found flagellates in fecal cultures from two persons that resemble the descriptions of *Enteromonas hominis*, but our preparations fixed in Schaudinn's fluid and stained by Heidenhain's iron-hemotoxylin method are not sufficiently clear to use for purposes of identification.

The methods of diagnosis commonly in use are as follows:

1. A small portion of the fecal material is mixed on a slide with a drop or two of normal saline solution, and spread out under a cover glass. The movements of active flagellates render them quite conspicuous when examined with relatively low magnification. Flagellates in the trophozoite stage that have ceased to move are more difficult to find, unless very numerous, even with the high dry objective. Cyst stages are known for all of the species listed above except *Trichomonas*



*hominis* and these can be distinguished from one another by shape, size and contents. The diagnostic characteristics of the trophozoites and cysts of these flagellates are stated in various recent publications, such as Hegner and Cort, 1921, and Dobell and O'Connor, 1921.

2. A similar fecal sample may be mixed with a solution of 5 per cent. potassium iodid in normal salt solution saturated with iodine. Material prepared in this way is particularly valuable for bringing out the internal structure of cysts, but of course this treatment renders the free forms immobile.

3. Eosin or neutral red in solutions of 1:10,000 are useful in determining the viability of cysts since dead cysts are stained by them and living cysts are not.

4. Fixation of wet smears in hot Schaudinn's solution, followed by Heidenhain's iron-hemotoxylin stain, has been found to give the most accurate results and has been employed by some investigators in doubtful cases.

5. Concentration methods such as that of Cropper and Row, modified by Boeck (1917), may give a higher percentage of positives.

The ease with which certain of these intestinal flagellates can be grown in culture, and the fact that the movements of trophozoites cease soon after the feces are passed, suggested the experiments recorded in this paper. The culture method might be expected to give favorable results in the case of *Trichomonas hominis*, a species whose cysts, if any, have not yet been discovered, and in the diagnosis of feces that have been passed for some time and hence contain no actively motile trophozoites, or in feces that contain such a small number of passive or active trophozoites that the chances of finding one in a smear are very slight. The successful cultivation of human intestinal flagellates is of quite recent date. So far all such cultures have contained bacteria which may serve as a necessary factor in the food supply of these flagellates.

*Chilomastix mesnili* was grown and maintained in culture for a period of four and one-half months by Boeck in 1920. He employed most successfully a medium consisting of one part of human blood serum and four parts of Locke's solution, plus 0.25 gr. dextrose per 100 c.c. A more limited growth and reproduction was obtained when horse or sheep serum was substituted for the human serum. The culture medium was distributed in 5 c.c. amounts in small test tubes. These were incubated at 37 C. over night and those which showed bacterial contamination the next day were discarded. A loop of feces containing numerous flagellates was washed in several successive samples of warm sterile normal saline solution. A few drops of this suspension were then transferred into several tubes of culture medium and incubated at 37 C. The next day transplants were made from the

positive tubes. Most of the flagellates were found feeding upon bacterial clumps at the bottom of the tube. No cysts were found in any of the cultures.

Various authors have cultivated *Trichomonas hominis* in various media. The most pronounced success, however, seems to have been attained by Hogue (1921 b), who carried on pure line as well as general stock cultures for several weeks. One of the media used by her was made by thoroughly shaking up a hen's egg in a flask with glass beads, and adding to it 200 c.c. of Locke's solution. This was heated over a hot water bath and kept in constant motion for 15 minutes. It was then filtered through cotton with a suction pump, tubed in 6 c.c. amounts, and autoclaved for 20 minutes at 15 pounds pressure. She also employed successfully an ovomucoid medium which will be discussed later. The tubes were inoculated and incubated at 35 C. In these media the *Trichomonas* appeared in greatest numbers on the 2nd and 3rd days. The addition of a few drops of human or sheep serum increased the number greatly. No cysts were found in any of her cultures of *Trichomonas*.

*Embadomonas (Waskia) intestinalis* was cultivated by the same author (Hogue, 1921 a) in the 2 media which she employed for *Trichomonas hominis*, in the Boeck medium described above, and in an ox bile salt medium. Both the motile and cyst forms appeared in the cultures.

Recently Wenyon (1922) has employed a modification of Noguchi's serum medium for the cultivation of a *Leptospira* and various protozoa, among them *Embadomonas intestinalis*, a species of *Embadomonas* from the guinea-pig, and a *Trichomonas* from a tortoise. This medium is prepared as follows:

To 270 c.c. of 0.85 per cent. saline solution ( $P_H$  7.6) are added 30 c.c. of ordinary 2 per cent. bacteriologic nutrient agar ( $P_H$  7.6). Ten c.c. of this mixture is placed in each test tube, and autoclaved at 120 C. When the tubes have cooled to 50 C., 20 drops of blood are allowed to drop into each tube from the margin of a rabbit's ear, previously shaved, sterilized with alcoholic iodine, and paraffined. After incubating for 24 hours the medium is ready for use.

This year in this laboratory we have found that *Trichomonas hominis* can easily be obtained in culture from an infected stool, and that the cultures can be carried along indefinitely in the Hogue modification of the ovomucoid medium. In addition, cultures of *Chilomastix mesnili* were readily obtained in this medium by inoculating infected material into the test tube and incubating at about 36 C. Transplants were made every two days for more than a month, and the flagellates were still growing vigorously when we ceased subculturing. We have never failed to get a heavy growth of *Trichomonas hominis* upon the first inoculation, but *Chilomastix mesnili* often appears only sparsely

in the first culture. However, upon inoculating a large amount of the first culture into a second tube of medium, swarming cultures almost always result.

A medium very similar to the Hogue formula was employed by Wherry (1913) in the cultivation of a free living amoeba. The difference between them lies chiefly in the fact that Wherry diluted the eggwhite to a somewhat less extent and used distilled water instead of normal saline solution. Wherry notes that egg white and water had been used previously by 1 or 2 authors as a culture medium for amoeba. However, the medium was first adapted to the cultivation of human intestinal flagellates by Hogue, as previously mentioned in this paper, and will be called the ovomucoid medium.

Preparation and use of the ovomucoid medium. We prepared our medium according to the simple directions given by Hogue. Briefly, the process is this: The whites of six hen's eggs are thoroughly shaken up with glass beads. This is added to 600 c.c. of 0.7 per cent. sodium chlorid solution. The mixture is cooked for 20 to 30 minutes over a boiling water bath, and is constantly agitated while cooking. The coarsest of the coagulated albumin is strained out by first passing through coarse cheese cloth. This is followed by filtering through cotton with a suction pump. The filtrate is then still quite opalescent. By means of a large pipette about 5 c.c. of this filtrate is put into each test tube, and the tubes are stoppered with cotton plugs. These are autoclaved at 15 pounds pressure for 20 minutes.

After autoclaving and allowing to stand a while, there should be a fairly clear, though slightly cloudy, supernatant fluid and a white flocculent precipitate. We have prepared this medium many times, but once such a precipitate did not form, the medium remaining colloidal. *Trichomonas* grew fairly well on this medium, but *Chilomastix* struggled along with difficulty. We should advise against using the medium for the cultivation of *Chilomastix* if this occurs, which is probably very seldom. If the medium is sterile it can be kept indefinitely, unless it is permitted to evaporate.

#### METHOD OF INOCULATION OF OVOMUCOID MEDIUM

We have found it very convenient to transfer the sample of feces to be tested for flagellates to the medium by means of a toothpick. Material is collected on the toothpick by taking a minute amount at random from different parts of the stool until an amount somewhat greater than the size of an apple seed is obtained. The toothpick is then dropped into a test tube of the medium by means of a pair of forceps, and the tube containing both fecal sample and toothpick is incubated at about 36 C.

The medium should be examined for flagellates about 24 hours after inoculation. If present they will be most numerous near the surface of



the medium. A large drop is looped off the surface onto a glass slide, and examined carefully under the low power of the microscope. We do not use a cover-glass on this drop since it spreads it out over a larger area and increases the difficulty in finding the flagellates if they are present only in small numbers. In order to determine the species of flagellate the examination should be made with the higher powers. However, if one is not quite familiar with the characteristics which distinguish the species, it is best to fix and stain cover slip smears made from the surface of the culture by the Schaudinn iron-hematoxylin method. The reader who wishes to acquaint himself with the morphology of the human intestinal flagellates should consult the papers listed at the end of this article.

In order to increase the certainty of the test for *Chilomastix*, it is best to pipette off the upper one-fourth of the apparently negative cultures and transfer this to a fresh tube of the medium. If *Chilomastix* is present it will then multiply very rapidly. No difficulty is experienced in obtaining growths of *Trichomonas* upon the first inoculation from a positive stool. Although we have encountered no *Embadomonas* (*Waskia*) infections, we see no reason why our method should not be useful in diagnosis of such infections, in view of Hogue's success in growing it on this medium. We found no mixed flagellate infections among the cases listed in this paper, but *Chilomastix*, *Trichomonas*, and *Embadomonas* have been grown in this laboratory all in a single tube in the ovomucoid medium, and media inoculated with fecal samples containing several of these species would no doubt favor the multiplication of them all, and hence aid in the diagnosis of such mixed infections.

The fact that *Trichomonas hominis* and *Chilomastix mesnili* could be so easily grown upon the ovomucoid medium suggested that we try it out on a small scale as a method of diagnosis of intestinal flagellates. The stools used were obtained through the kindness of Dr. C. B. Ensor from the Mount Hope Retreat insane hospital, from the Johns Hopkins Hospital with the assistance of Dr. C. G. Guthrie, and from a member of the school of hygiene and public health who carried an infection of *Chilomastix mesnili*.

The number of specimens examined, source of material, number found positive by the routine examination of smears, and number found positive by the culture methods are given in the following table:

TABLE 1.—COMPARATIVE RESULTS OBTAINED IN THE DIAGNOSIS OF INTESTINAL FLAGELLATES BY THE SMEAR AND CULTURE METHODS

Number of Specimens	Source of Material	No. of Positives by Smear Method	No. of Positives by Culture Method
43	Mount Hope Retreat.	0	5
67	Johns Hopkins Hosp.	2	3

Table 1 shows that the stools obtained from the Mount Hope Retreat insane hospital were all negative when examined by the smear method, but that 5 were found to be positive when inoculated into cultures. The flagellates found were 3 cases with *Trichomonas hominis* and 2 with *Enteromonas hominis* (?). Thus 5 positives out of 43 samples were overlooked by the smear method but discovered by the culture method.

It is of interest to note with respect to the material from the Johns Hopkins Hospital that in both cases in which flagellates appeared in the smears, one with *Trichomonas hominis* and the other with *Chilomastix mesnili*, they also appeared in the corresponding culture tubes.

The presence of a *Chilomastix mesnili* carrier in our department made it possible to make several examinations at intervals of the feces of a person known to be infected. Smears and cultures were made from fresh stools on the following dates and trophozoites were recorded as indicated.

Date	Smear Method	Culture Method
1. December 5, 1921.....	Positive .....	Positive
2. January 29, 1922.....	Negative .....	Positive
3. February 4, 1922.....	Negative .....	Positive
4. February 6, 1922.....	Negative .....	Negative
5. February 12, 1922.....	Negative .....	Negative
6. February 21, 1922.....	Positive for cysts.....	Positive
7. March 6, 1922.....	Negative .....	Positive
8. April 11, 1922.....	Positive .....	Positive
9. April 23, 1922.....	Positive .....	Positive
10. May 4, 1922.....	Positive .....	Positive

These data show that active flagellates were found by the routine smear method on 4 out of 10 examinations, but by the culture method on 8 out of 10 trials, a difference very much in favor of the culture method.

It is well known to those who have followed the course of infections with intestinal flagellates that sometimes no specimens can be found in the feces; at other times cysts only or trophozoites only are present; and sometimes both cysts and trophozoites appear in the stools of a carrier. The numbers of these, when present, likewise differ markedly at different times. The correct interpretation, therefore, of the data presented above probably is as follows. Smears were found to be positive for trophozoites on dates 1, 8, 9 and 10 because a rather large number of these were present. The smears were found negative for trophozoites on dates 2, 3, 6 and 7, because only a very few were present. Trophozoites probably appeared in the culture on date 6 because there were a few trophozoites in the sample, since the cysts

that were present are not known to develop into trophozoites in culture medium.

Efforts were made to determine how long after defecation it is possible to obtain positive cultures with *Chilomastix mesnili* and *Trichomonas hominis*. Accordingly, smear examinations of stools known to be positive for *Chilomastix* were made at various intervals; and cultures were made at the same time, with the following results:

*Chilomastix mesnili*

Time Stool Was Passed	Time of Examination and of Culture	Results of Smear Examination	Results of Culture
May 4, 7:30 p. m..	May 4, 7:30 p. m..	Positive .....	Positive
May 4, 7:30 p. m..	May 5, 8:00 a. m..	Positive—few alive..	Positive
May 4, 7:30 p. m..	May 5, 1:30 p. m..	Negative .....	Positive
May 4, 7:30 p. m..	May 5, 3:30 p. m..	Negative .....	Negative

These results show that living trophozoites of *Chilomastix mesnili* may appear in cultures after they can no longer be found in the stools by the smear method. Trophozoites of this species were not found in smears after 12½ hours but appear in cultures after 18 hours.

Several other experiments indicate that the trophozoites of *Chilomastix* remain alive in stools kept at room temperature for less than 24 hours. For example: (1) fecal material from a stool passed on Jan. 29, 1922, was inoculated into tubes immediately which later contained trophozoites. Other tubes inoculated when the stool was 24 and 48 hours old, respectively, were negative. (2) Another stool containing trophozoites was obtained on April 23, 1922, at 7:10 p. m. Cultures inoculated at 8 p. m. on April 23, and at 8 a. m., 10 a. m., 12 n., and 3 p. m. on April 24 were all positive, thus showing that these organisms were still viable 19 hours after defecation. Cultures made later were all negative.

One of our experiments indicates that the trophozoites of *Chilomastix* remain alive longer in fecal material kept at room temperature than in fecal material placed in an incubator heated to 37 C. A stool containing both active flagellates and cysts was obtained on April 11 and divided into 2 portions, 1 portion being placed in a moist chamber at room temperature (27 C.) and the other in the incubator at 37 C. Cultures from these were made 12 hours later; those inoculated with material that had been in the incubator were negative, the others, inoculated with the material kept at room temperature, were positive.

Smear examinations were made and culture tubes inoculated with material from a stool containing large numbers of *Trichomonas hominis* kept in a moist chamber. The results of this study are as follows:



*Trichomonas hominis*

Time Stool Was Passed	Time of Smear Examination and of Culture	Results of Smear Examination	Results of Culture
April 29, 9:00 a. m..	April 29, 12:15 p. m.	Positive .....	Positive
April 29, 9:00 a. m..	April 29, 11:00 p. m.	Positive .....	Positive
April 29, 9:00 a. m..	April 30, 12:00 N.	Positive .....	Positive
April 29, 9:00 a. m..	April 30, 10:30 p. m.	Positive (few).....	Positive
April 29, 9:00 a. m..	May 1, 8:00 a. m..	Negative .....	Positive
April 29, 9:00 a. m..	May 1, 9:00 p. m..	Negative .....	Positive
April 29, 9:00 a. m..	May 2, 7:00 a. m..	Negative .....	Positive
April 29, 9:00 a. m..	May 2, 4:00 p. m..	Negative .....	Positive
April 29, 9:00 a. m..	May 3, 8:00 a. m..	Negative .....	Negative
April 29, 9:00 a. m..	May 3, 4:00 p. m..	Negative .....	Negative

As the above table shows, no motile trophozoites were found by the smear method 47 hours after the stool was passed, but positive cultures were obtained when the feces were 79 hours old.

It will be seen from these data that *Chilomastix mesnili* is not nearly so viable in stools as is *Trichomonas hominis*. This fact may have some bearing on the apparent absence of cysts in the life cycle of the human *Trichomonas*, the great viability of the trophozoite of this species making it possible for it to gain access to new hosts without the aid of cysts.

Perhaps an extremely painstaking and prolonged examination, such as would not be practicable in actual diagnostic work, would have disclosed the presence of a very few organisms still alive in the specimens recorded as negative. However, the fact that they were readily cultivated from these old stools shows a decided advantage for the culture method.

Our data also furnish evidence that trophozoites of *Chilomastix* do not develop from cysts in the types of cultures we used since viable cysts were present in the stools at times when negative cultures were obtained.

## SUMMARY

1. The trophozoites of 4 species of human intestinal flagellates may be grown in a simple culture medium; these are *Chilomastix mesnili*, *Trichomonas hominis*, *Embadomonas intestinalis*, and *Enteromonas hominis*.

2. The simplest and most practicable culture medium is the ovomucoid, consisting of a mixture of white of egg and 0.7 per cent. normal saline solution.

3. The superiority of the culture method over the smear method for diagnostic purposes is indicated by the following data: (1) Fecal specimens were obtained from 110 individuals. Trophozoites were found by the smear method in only 2 of these; whereas 8 of the culture

tubes were positive. (2) Stools from a *Chilomastix* carrier were examined on 10 days at various intervals between Dec. 5, 1921, and May 4, 1922. Smears from these were positive for trophozoites on 4 occasions, but the cultures contained trophozoites on 8 days. (3) Trophozoites of both *Chilomastix* and *Trichomonas* may be obtained from stools by the culture method after they can no longer be found by the smear method.

4. Trophozoites of *Trichomonas* appear to be more viable than those of *Chilomastix*. Positive smears of *Trichomonas* were obtained 37½ hours after a stool was passed and of *Chilomastix* only 12½ hours after defecation. *Trichomonas* was obtained in culture from a stool 79 hours old, whereas *Chilomastix* could be cultivated only from 18 to 19 hours after the stool was passed. It is suggested that the great viability of the trophozoite of *Trichomonas* may enable this species to gain access to new hosts without the aid of cysts.

5. Cysts of *Chilomastix* did not give rise to trophozoites in our culture media.

6. The preliminary study made of this subject lead us to conclude that routine stool examinations by the culture method are not only possible but also practicable and more efficient than those made by the smear method. The culture method is indicated, especially when the organisms are likely to be very few in number or under circumstances that make it impossible to obtain the stools for examination when fresh. It is particularly valuable for the diagnosis of *Trichomonas hominis* which has no known cyst stage to aid us in determining its presence. We hope to be able to test this method further in the near future.

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# COUNCILMANIA LAFLEURI NOT A NEW AMOEBA

HERBERT GUNN

The description by Kofoid and Swezy (1921) of a supposedly new amoeba occurring in the intestinal tract of man and hitherto classified as *Entamoeba coli* has necessitated a critical study of this class of cases. Kofoid and Swezy claim this amoeba is pathogenic and that it differs from *Entamoeba coli* in a number of characteristics in both the free and encysted stages. During the past year I have had eight cases, pronounced by Dr. Kofoid's laboratory as *Councilmania lafleuri*, which were studied carefully in the free and encysted stages, comparing them for checking purposes with specimens from *Entamoeba coli* infections which also had been pronounced by Dr. Kofoid's laboratory as *Entamoeba coli*.

Kofoid and Swezy give the following as the available diagnostic characteristics, and I will consider them seriatim. In the original they are as follows:

<i>Councilmania lafleuri</i>	<i>Endamoeba coli</i>
Free Stage	
1. Very active, pseudopodia thrust out suddenly, ectoplasm sharply separated from endoplasm.	1. Sluggish, ectoplasm not sharply separated from endoplasm.
2. Red blood corpuscles ingested readily.	2. Red blood corpuscles not ingested normally.
3. Peripheral chromatin in a thin layer, karyosome large, eccentric, with halo, or often seen in premitotic condition with chromatin dispersed in granules in a sphere, ring, or skein without halo and often central.	3. Peripheral chromatin in a thicker layer, karyosome small, spherical, with halo, generally eccentric.
Encysted Stage	
4. Cyst wall very thick.	4. Cyst wall thin.
5. Spheroidal, ellipsoidal or asymmetrical, less often spherical.	5. Generally spherical.
6. Less readily stained.	6. More readily stained.
7. Glycogen body more resistant to iodine.	7. Glycogen body stains readily in iodine.
8. Nuclei with little peripheral chromatin and large, generally central or but slightly eccentric, dispersed karyosome.	8. Nuclei with more peripheral chromatin and small, eccentric, massed karyosome.
9. Chromatoidal bodies less acicular in early stages, fasciculate, massed centrally in later stages and contributing to chromophile buds.	9. Chromatoidal bodies more distinctly acicular, with less central massing and no relation to segregation of chromophile cytoplasm.
10. Chromophile ridge forms a bud through a pore in the cyst wall, which detaches uninucleate amoebulae.	10. Budding unknown.

1. Any attempt to pass on amoebae, based on motility and appearances of pseudopodia or differentiations of endoplasm and ectoplasm, is liable to great error. A careful study of a case of either *histolytica* or *coli* infection will show under different conditions very great differences in the motility and appearance of parasites. While it is generally taught to the contrary, it is nevertheless a fact that *Entamoeba coli*, under favorable conditions, may exhibit the same active motility that is considered so characteristic of *Entamoeba histolytica*. I could obtain the same motility in check cases that was seen in the so-called Councilmania.

2. In none of the parasites that I examined were red blood cells present. It is possible that Kofoid and Swezy were dealing with mixed infections with *Entamoeba histolytica* as suggested by Wenyon (1922), or that vacuoles were mistaken for red cells, an error readily and frequently made.

3. In the free state no differences could be noted as far as the nucleus could be studied in unstained specimens.

4. There appeared to be no difference in the thickness of the cyst walls in the Councilmania and check cases.

5. No difference in contour could be demonstrated between the cysts of Councilmania and the *Entamoeba coli* check cases, nearly all being spherical. It is significant that Kofoid and Swezy describe 12 per cent. of the cysts of Councilmania as being asymmetrical while the cysts of *Entamoeba coli* were generally spherical. In my cases asymmetrical forms were readily produced by pressure on the cover slip.

6. It is stated by Dr. Kofoid that his attention was drawn to the Councilmania cysts by the fact that they usually took the hematoxylin stain reluctantly and that they were often found as unstained bodies in the preparation. This I could not verify, the cysts taking the stain, both iodine and hematoxylin, as readily as the check cases, in fact the first three cases that were pronounced Councilmania in my series apparently took the stain more readily than usual. Just as many unstained cysts were seen in the *coli* check cases as in the Councilmania. It is not an infrequent observation to encounter amoebic cysts which are difficult to stain and it is noted in other species as well as *coli*.

7. There was no difference in the reaction to iodine of the glycogen body in the Councilmania and *Entamoeba coli* check cases.

8. There was no difference in the peripheral chromatin of the nuclei. There was no difference in the appearance of the karyosome in the Councilmania and *Entamoeba coli* cases. The karyosome described by Dr. Kofoid in the Councilmania differs somewhat from the description usually given of the karyosome in the *coli* but I found examples of this



same karyosome in all cases classified as *coli* in my checks. Dr. Kofoid's pictures of the karyosome are, I believe, the best I have seen.

9. There appeared to be no difference in the chromatoidal bodies of the *Councilmania* and of the *Entamoeba coli* check cases.

10. Nothing in the slightest manner resembling a budding could be seen in fresh specimens, specimens stained in iodine eosin, in iodine or in hematoxylin, although several thousand cysts were scrutinized very carefully for it.

Dr. Kofoid states that this budding cannot be induced by heat and in this he may be right. He also states "It is not due to trauma and cannot be produced by pressure." With this I take exception. By pressure on the cover slip I have produced budding in practically all of the stages described by Dr. Kofoid in his paper. In some cysts the entire contents were evacuated, in others one or two nuclei. In some the nuclei were seen escaping or partly extruded. Dr. Kofoid states:

"The intracystic ridge which precedes and attends the early stages of the formation of the bud first appears as a chromophile deeply staining tract which becomes an elevated ridge or keel of varying width which may run half way round the cyst or even further within the wall. It may be a dark narrow thread or a blunt process or a broader ridge. It is generally direct in its course, but is sometimes bifurcated at one end. In optical section it forms a distinct elevated ridge on the contour of the cytoplasm within the cyst wall. . . . Not all cysts have chromatoidal bodies and not all ridges are deeply chromophile, but they are generally more deeply stained than the cytoplasm."

This deeply stained ridge was very clearly brought out in all the specimens where budding was produced by pressure and it resulted of course from the entrance of the stain into the creased wall of the ruptured cyst. This was definitely demonstrated in several instances where the cyst originally would not take the iodine eosin or iodine stain. As soon as the cyst wall was ruptured by the proper degree of pressure the contents of the cyst became immediately deeply stained, the deeply stained ridge described above became prominent and budding occurred.

When I read Dr. Kofoid's article it seemed to me incredible that during the years I have been working with this subject, I had completely overlooked a process so striking as the budding that he describes. However, it often happens that unless one's particular attention is drawn to certain subjects, more or less detail may readily be overlooked. This I know to be so in regard to the character of the karyosome in the *Entamoeba coli*, which my studies started by Dr. Kofoid's article have shown me.

An analysis of my observations would lead me to the following conclusions; first, that there is no foundation for considering *Councilmania lafleuri* a new amoeba; second, that the authors were dealing entirely with *Entamoeba coli*, being led into error by an incorrect interpretation

of staining reaction, by according too much weight to characteristics of amoebae in the motile state, and by the production of artefacts in the preparation of specimens.

I must agree with Wenyon (1922) when he adds *Councilmania lafleuri* to the long list of synonyms of *Entamoeba coli*.

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## ACTINOMYCOSIS IN A FOSSIL RHINOCEROS

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The transmission of the ray fungus, *Actinomyces*, through grass, straw, chaff, the beards of wheat to cattle and the ensuing pathology of the oral region, the lungs and other parts of the body are well known, and there are several excellent reviews of these matters. While cattle are most susceptible, as seen in the numerous cases of "lump-jaw" and other actinomycotic infections, yet the fungus is known to cause a similar pathology in man, the horse, elephant, dog, pig and other mammals. So far as I am aware no infection of the rhinoceros has been reported, so the presently described case is the more interesting.

Mr. Harold J. Cook, of Agate, Nebraska, has recently loaned me for study the jaw of a fossil rhinoceros, *Aphelops*, from the Snake Creek Beds, Pliocene, of the northwestern part of Nebraska. Matthew and Cook have reported on this fauna, which is represented by abundant though fragmentary remains. The present specimen consists of all the right mandibular ramus and a portion of the left, which is diseased. The pathologic area has all the appearances of an *actinomycotic osteitis*, and while one must use due caution in diagnosing fossil lesions, yet the present case is remarkably similar, in all its appearances, to modern examples of "lump-jaw."

The preserved portion of the lesion involves the alveolus of the left large incisor tooth and is, apparently, the oldest and so far the only fossil neoplasm of a definite actinomycotic nature. Some years ago I suggested that a swelling in the jaw of a fossil horse, *Meryohippus campestris*, might be due to actinomycosis in its early stages, yet there was no definite indication of the nature of the infection externally. The value of the specimen as a type fossil forbade dissection. This leaves the fossil rhinoceros jaw as the most suggestive case yet seen among fossil vertebrates.

Direct comparison of the fossil lesions with a modern example of "lump-jaw" reveals a number of similarities. In the fossil neoplasm, most of which unfortunately is lost, the exterior is relatively firm, while the interior of the tumor-mass is mealy in appearance with numerous necrotic sinuses. The sinuses channel out to the surface of the jaw and one had formed into the alveolus. Apparently near the center of the mass nearly all traces of osseous structure was lost, due to the destructive activity of the ray fungus. There is no indication of healing and doubtless the infection was active at the time of death of the animal, suggesting that the infection, then as now, was of long duration.

# TRICERCOMONAS INTESTINALIS AND ENTEROMONAS CAVIAE N. SP. AND THEIR GROWTH IN CULTURE

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*Enteromonas hominis* is the name given by da Fonseca (1915) to a new flagellate of the intestine of man, a very small organism possessing three anterior flagella, two of which are directed forward and one, somewhat longer, directed posteriorly but not adherent to the surface of the body. Wenyon and O'Connor (1917; 1919) described a somewhat similar small flagellate; however, it has four anterior flagella, three directed forward and one recurrent, attached to the body surface for a part of its length. The cysts of *Tricercomonas* were also described but da Fonseca reported no cyst for his organism.

Dobell (1921) considers that these two flagellates are probably the same and accepts Wenyon and O'Connor's description, their name, however, giving way to *Enteromonas hominis* da Fonseca. He tentatively identifies with this species also *Trichomastix hominis* Chatterjee (1917), *Diploccercomonas soudanensis* Chalmers and Pekkola (1919), and *Enteromonas bengalensis* Chatterjee (1919), all of which bear slight variations in their descriptions.

Recently the writer has had occasion to observe a small flagellate which is apparently identical with *Tricercomonas intestinalis* Wenyon and O'Connor and to make some comparisons between it and a flagellate from the guinea-pig which resembles closely *Enteromonas hominis*.

The human flagellate comes from the stools of a woman, in which it is associated with *Chilomastix*, the host showing no intestinal symptoms referable to the presence of the organism. The flagellate was not seen in the material from a duodenal drainage and consequently is to be considered a lower intestine inhabitant. It probably occurs in the large intestine as was the case of the flagellate from the guinea-pig. It occurred in considerable numbers in the fresh stool from which it disappeared within 24 hours.

It varied in size from about 4 to 7 or 8 microns in its longest diameter, it being almost rounded or slightly ovoid. It is very active in fresh preparation, swimming jerkily and rotating at the same time. The body is alveolated and vacuolated and contains bacteria. It anchors itself by a caudal process as is the habit of other flagellates when feeding. The anteriorly directed flagella move rapidly and are not to be counted with certainty in an active stage. At times they can be seen



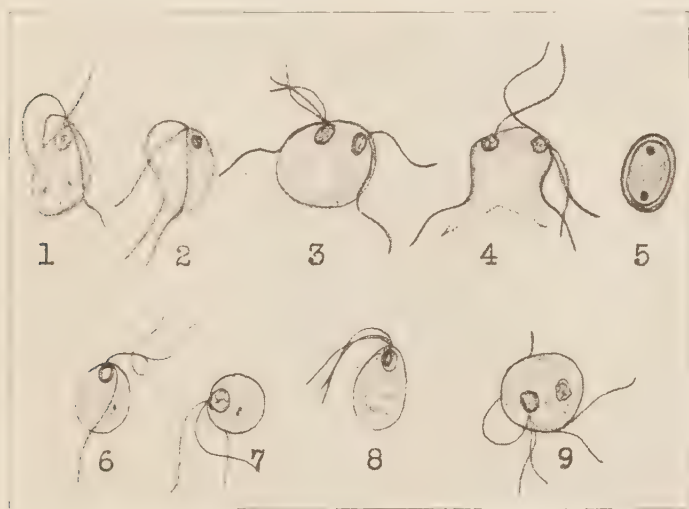
to come from a minute knob, apparently the blepharoplast. There is a recurrent flagellum which arises at the same place, passing posteriorly over the surface of the body to which it is adherent for about three-fourths of the body length, terminating in a free extremity from the posterior quarter. In motion there is a slight protoplasmic projection where this flagellum leaves the body. The nucleus is not visible in the unstained. Only a few cysts similar to those described by Wenyon and O'Connor have been observed. These were ovoid, slightly smaller than the active organism, having a definite cyst wall enclosing the organism which contained a nucleus at each end in the stained specimens. Further development of the cyst has not been seen in this case. Proper observation of the flagella has been difficult in direct preparations even when stained. In thin films made from cultures they have been stained with Wright's blood stain. There are three of about equal length directed forward, the one recurrent being slightly longer. The nucleus may be properly observed in direct preparations as well as in specimens from culture stained by the iron-haematoxylin method. It is ovoid and situated near the origin of the flagella. Usually it shows a distinct karyosome but in the dividing the chromatin is loosely distributed through the nucleus.

The organism grew well in ascitic fluid diluted with 4 parts of 0.9 per cent. sodium chloride solution at 37 C. for four days but transplants were not successful. It lived in diluted blood serum for a few days but apparently did not multiply. In the ascitic fluid the size ranged from about  $3\mu$  in the young to about  $10\mu$  in the large dividing forms. Activity was about the same as in the fresh stool and the number increased very materially during the second 24 hours. No encystment was observed. Division, although difficult to follow and not completely observed, is apparently by longitudinal splitting. The chromatin becomes distributed through the nucleus and it divides into two before the splitting of the body commences. Whether the nuclear division is mitotic has not been made out. The two halves are equipped with flagella before the body division occurs. Whether there is a full quota of 3 anteriorly directed flagella on each at first is uncertain, but at least 1 of the 2 has 3 and the recurrent fully developed before body division is completed. The other has the recurrent flagellum but only 1 anteriorly directed has been observed. Multiple division may occur as some forms with 4 nuclei were seen. The small size of the organism makes it very difficult to follow accurately the nuclear changes and the process of division.

#### *Enteromonas of the Guinea-Pig*

A flagellate somewhat similar to the above and closely resembling da Fonseca's *Enteromonas hominis* has been encountered in the guinea-pig. The size, motion, shape, nucleus, cultural qualities, and general

appearance of the organism correspond to those given above for the *Tricercomonas*. However, there appear to be only two anteriorly directed flagella and the recurrent one does not appear to be adherent to the body, at least it is not always so. It often lies back over the body but frequently is distinctly unattached and sometimes occurs in a group with the other two. Multiplication takes place in culture similarly to the case of the *Tricercomonas*. Encystment has not been recognized. This flagellate appears to be at least closely related to da Fonseca's organism and it is proposed to call it *Enteromonas caviae* n. sp.



Figs. 1-5.—*Tricercomonas intestinalis*. Figs. 1 and 2 composite drawings of specimens from culture, Wright's stain used for the flagella and iron-hematoxylin for the nucleus. Figs. 3 and 4 dividing forms from culture, Wright's stain. Fig. 5, cyst.

Figs. 6-9.—*Enteromonas caviae* n. sp. Composite drawings as above.

#### Discussion

From the above observations it seems at least doubtful that *Enteromonas hominis* and *Tricercomonas intestinalis* are the same. The observations of *Tricercomonas intestinalis* by Wenyon and O'Connor are substantially confirmed in the case of the human flagellate and its geographic distribution is widened. The occurrence of the similar flagellate in the guinea-pig does not, of course, prove that the observations reported by da Fonseca on his flagellate in man are correct, but the observations here recorded indicate that there is such an organism as he describes and lends support to the indication that *Enteromonas* and *Tricercomonas* are distinct. At any rate the evidence is considered

sufficient to allow *Tricercomonas intestinalis* to be retained for the present as the intestinal flagellate described by Wenyon and O'Connor.

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NOTES ON *EMBADOMONAS SINENSIS*.  
FAUST AND WASSELL 1921 \*

ERNEST CARROLL FAUST

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*Embadomonas sinensis* was recovered from diarrhoeic stools of Chinese patients in the Church General Hospital, Wuchang, in July, 1921, and described by Faust and Wassell under that name (1921: 543). During a month's intensive study of 57 medical cases, 9 stools were found to contain this flagellate. In all of these cases save one, vegetative or cystic stages of *Entamoeba dysenteriae* had been previously demonstrated. It has since been found in the same hospital, but as yet has not been demonstrated in other localities in China. Normal quiescent individuals are seen in Figures 1 to 4.

The living organism is extremely active. In moving forward it performs a smooth spiral glide, at which time the animal is oval-elongate, and on careful observation is found to be eugleniform (Figs. 5-7). This is obviously due to the extreme plasticity of the protoplasm, for on becoming quiescent, especially at periods of feeding, the organism assumes an obovate outline, very similar to that of the related species, *Embadomonas intestinalis* (Wenyon and O'Connor). At times, however, quiescent forms (Figs. 8-10) become elongate and attenuate, particularly in the posterior half of the body, but on stimulation they resume once more the normal obovate form. The species under consideration tends, therefore, to assume a characteristic shape and form, although the protoplasm lends itself readily to change under the proper conditions. In motile forms the body has an average measurement of  $14\mu$  in length by  $4.2\mu$  in transverse diameter. Quiescent forms have a mean average of  $10\mu$  in length by  $7\mu$  in width. Attenuated individuals may reach a body length of  $20\mu$ . Certain strains are much larger than others. On the whole, it seems probable that the average measurements of this species are considerably larger than those of *Embadomonas intestinalis*.

In general, the specific characters of *E. sinensis* agree with those of *E. intestinalis*, although differences are apparent. In the first place, there is not the marked differentiation between anterior and posterior flagella, which probably accounts for the smoother movement of the Chinese species. These flagella are, however, definitely specialized in

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\* Contribution from the Parasitology Laboratory, Department of Pathology, Peking Union Medical College.



direction and probably in function, although as far as I have observed their movements are synchronized. They stain readily with Donaldson's eosiniodide preparation. In the quiescent organism the cytostome is wide and pouch-like; in the attenuate individual it is an elongate gullet, which may at times become slightly spiralled to conform to the body form. There is a blepharoplast at the base of each of the flagella. These blepharoplasts lie an appreciable distance from the nucleus. I have not been able to demonstrate a neuromotor connection with the latter organ. The nucleus is somewhat smaller than that of *E. intestinalis*. Excretory vacuoles are common but the general consistency of the protoplasm is smooth and homogeneous. The polar view of the organism at rest is round (Fig. 12).

The organism divides by longitudinal fission, with separation of the daughter elements at the posterior end, even while the anterior organelles are still in the process of division. After separation the daughter cells are at first pyriform, but soon become actively motile and assume an elongate shape.

Small ovate-elongate cysts, which were found in the same stools, apparently belong to this species. They measure 3 by  $6\mu$  and are most abundant after the vegetative forms have disappeared from the stools.

*Embadomonas intestinalis* has been found in Alexandria, Egypt, and in overseas and home-service troops of the United States in New York, as well as in natives in the United States who have no contact with imported cases. *E. sinensis* has been found only in native Chinese at Wuchang. In both instances the infections are from diarrhoeic stools or those containing *Entamoeba dysenteriae* or *Trichomonas hominis*. In amoebic dysentery they appear several days after the critical period and are found to grow more rapidly at room temperature (30 C.) than at body heat. However, I have noted particularly that they have been derived from the stool and not as a contamination from bed-pan or specimen box. It seems more than likely, therefore, that the organism is not itself primarily a parasite, although large numbers of the species in the stool may give rise to diarrhoea. Nevertheless, it is significant that the species occurs in patients suffering from amoebic dysentery. It seems safe to assert, then, that it fits into the environment and same organic cycle as *Entamoeba dysenteriae*. Even in questionable cases where *Ent. dysenteriae* has not been demonstrated in the stool, the presence of Embadomonads may be indicative of amoebic infection.

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EXPLANATION OF PLATE VI

Figs. 1-4.—*Embadomonas sinensis*, quiescent forms.

Figs. 5-7.—Actively motile individuals.

Figs. 8-11.—Elongate individuals.

Fig. 12.—Polar view of quiescent individual.



# SOCIETY PROCEEDINGS

## THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The fifty-fourth meeting was held at George Washington University, on Oct. 21, 1921.

Dr. Hall presented a paper on "Sterile Tapeworms in Unusual Hosts."

Two entire specimens of tapeworm and one fragment collected from the goat at Antigua, British Indies, were sent to the federal Bureau of Animal Industry by Dr. H. Goodwin in May, 1920. These worms were evidently anoplocephaline tapeworms, but it was impossible to determine the genus as the genitalia were inadequately developed even in specimens showing the narrowing of the segments which precedes the separation of gravid segments. The complete worms were 3.5 and 7.5 cm. long and the fragment 10 cm. long. Rudimentary genital canals and ovaries were present in many segments, but the relation of the genital tubes to the excretory canals could not be determined. The fact that these tapeworms were sterile and possessed only rudimentary genitalia suggests that the host is an unusual one, and it is of some interest to correlate this finding with other findings along this line.

Dr. Goodwin sent also a tapeworm collected from a pig at Antigua. This worm is also an anoplocephaline tapeworm, but the genitalia are so rudimentary that identification is impossible. Apparently the sterility is associated with development in an unusual host, since no intestinal tapeworms are normally present in swine. Kholodkowsky has reported *Thysanosoma giardi* from swine, Stiles has reported three cases of tapeworms from this host and Oettle one. As Stiles has noted, records of this sort appear on first sight to be open to suspicion since swine may have eaten these tapeworms a short time before being killed and examined, but evidently swine may occasionally serve as hosts in cases of accidental infestation. Where the worms are sterile, this fact favors the idea that they developed in this host, the incompleteness of development being associated with the unsuitable environment of the unusual host. Douthitt has described *Andrya translucida* from *Geomys bursarius* and notes that in his material most of the terminal segments were devoid of eggs. He concludes that the tapeworm was not found in its usual host and that the comparative sterility is due to the unusual environment. A large amount of evidence indicates the occurrence of nematodes of various sorts in unusual hosts, with a concomitant sterility or imperfect development.

Mr. Augustine presented a note by Cort and Augustine on apparatus used in making a soil survey in the island of Trinidad during their study of the distribution of human hookworm larvae.

Dr. Cort presented a note by Cort and Augustine on *Observations on hookworm larvae in the soil*.

(See a series of papers on Investigations on the Control of Hookworm Disease, Amer. Jour. Hyg., v. 1, 2; 1921, 1922.)

Dr. Hall noted that the wash of rain on hill pastures apparently resulted in keeping them more or less cleaned of worm infestation, at the same time increasing the infestation in the valley pastures, and that shepherds for centuries had noted that hill pastures were especially well adapted to sheep, since they appeared to be more healthy and thrifty on such pastures. Dr. Darling spoke in confirmation of the same idea, noting that in the case of the human hookworm it had been observed that there was less infestation among persons living on a hillside than among those living in the valley.

Dr. Curtice noted that in a study of sheep parasites he had found that even where there was abundant vegetation in the valleys the sheep preferred to graze on the higher hillsides, cropping them close and leaving the lower vegetation uncropped. He noted that at certain intervals after a rain the count of worm eggs present in the manure increased. It had been found that the con-



trol of these parasites by routine anthelmintic treatment once a month was superior to pasture rotation accompanied by desultory treatment. The results of worm control were manifest in increased gain in weight and wool production. He is now testing treatment at intervals of 3 weeks and believes that persistent treatment in man promises to be one of the best control measures.

Dr. Cort noted that 5 percent formaldehyde killed free-living nemas found in soil, but did not kill hookworm larvae. Baermann was confused in regard to larvae found without sheaths. Dr. Ransom stated that putrefactive bacteria were among the most important enemies of hookworm larvae. Dr. Cort noted that some free-living nemas were also detrimental to the larvae. He added that the use of ashes in cultures aided in keeping down putrefactive bacteria. Dr. Bartsch noted that one is advised to wear gloves in working in soil in the tropics, but in his experience in collecting molluscs gloves are annoying and are usually soon discarded. He had never become infected under these conditions, a fact which bore out Dr. Cort's conclusions in regard to the limitation of soil infestation.

Dr. Ransom noted in connection with the longevity of larvae in the tropics, that tick larvae live longer at lower temperatures than under higher, within certain limiting ranges. Dr. Shillinger asked in regard to tests of soil acidity in connection with infestation with hookworm larvae. Dr. Cort reported that this was not tested, though work along this line was planned. Dr. Hall called attention to the test reagents for this purpose devised by Wherry. In this connection, Dr. Cort reported that in one area with a high degree of soil pollution, where there was considerable shade and much humus, the soil infestation was very light.

Dr. Cort noted that hookworm larvae must die rapidly during the dry season and suggested that this might prove to be the best time to push hookworm treatments, thus cutting off soil infestation at its source. It would appear that a second treatment should follow the first after an interval of 6 weeks and a third treatment after an interval of 3 weeks, the first interval being correlated with the 6-weeks' duration of the life of larvae in soil as observed in Trinidad. Dr. Curtice reported that in the experiments on sheep parasites carried on at Vienna, Virginia, by the Bureau of Animal Industry, no hookworms had been found in the sheep for four or five years, due, apparently, to the control measures employed.

Dr. Cobb presented the following notes:

*How Certain Apogeotropic Nemas Climb on a Dry Surface*

Certain nemas parasitic on and in some of the dung beetles (*Aphodius fimetarius*, etc.) are able to crawl on dry surfaces by virtue of an oily secretion poured out through the mouth. These nemas do not wet with water. They lie on its surface and tend to float together in adherent groups, apparently by virtue of an external coating. On treatment with osmic acid these nemas darken, indicating the existence in them of such substances as fatty and oily compounds which react in this manner. Mounted alive in water, under slight pressure these nemas extrude and withdraw at the mouth opening a non-miscible substance having a refractive index different from that of water. A chemically clean cover glass over which these nemas are allowed to crawl will display traces of this substance by showing the darkening reaction with osmic acid, the darkened substance not being soluble in water. This evidence makes it extremely probable that these nemas utilize this secretion in climbing dry surfaces. Whenever these nemas are placed in a drop of water on a dry slanting or perpendicular surface, they promptly leave the water and begin to crawl upward on the dry surface, whether in the light or in the dark.

*Howardula benigna*; a Nematode Parasite of the Cucumber Beetle (*Diabrotica*)

The occurrence of this nema has already been reported. The beetles it infests do great damage, occurring over the entire country. The nema is found on an average in 20 percent of these beetles. In the vicinity of Washington it was found this year in about 50 percent. There are no males, this

being a syngonic form. The larvae leave the beetles with the eggs, from 6 to 50 larvae to each egg. As the grub hatches, the nemas bore into it. This nema appears to be a control factor of great economic importance and is one that is already available. Supplies of the nema are already being sent out to infested districts as an aid in control.

Dr. Cobb also reported a larval nema from the dung beetle, *Aphodius fimetarius*, and stated that he had found amphids in this worm. Dr. Ransom identified the worm as a larva of *Gongylonema*. This is the first time that amphids have been reported definitely under that name from parasitic nemas. They appear to be the organs commonly called the lateral papillae in parasitic forms. In comment on the secretion poured out by nemas in climbing over a dry surface, Dr. Hall noted Schewiakoff's theory of gregarine movement, to the effect that the translatory movement was due to an extruded gelatinous thread which flowed posteriorly and hardened, thereby pushing the gregarine ahead, and to Crawley's comments on this theory.

The fifty-fifth meeting was held at the School of Hygiene and Public Health of Johns Hopkins University on November 19, 1921.

Dr. Bartsch presented a note in regard to the mollusc genus *Blanfordia*. Robson has recently shifted the genus *Nosophora* to *Hypsobia*, but as *Hypsobia* is antedated by *Blanfordia*, the latter must stand as the correct name of the genus. Dr. Cort commented on the importance of this snail as a host of *Schistosoma japonicum* and stated that he had submitted specimens of the mollusc, furnished him by Dr. Katsurada, to Robson for determination.

Dr. Simon presented a note on the occurrence of *Loxogenes arcanum* in *Rana clamitans* in Nova Scotia. A frog had been found anemic on examination and on further study tumors were found near the pylorus. From these tumors flukes belonging to the species named could be squeezed out. The tumors were common in frogs in Nova Scotia. The fluke was discovered in the liver of frogs in Ontario by Stafford in 1900. The same year Nickerson found it in cysts near the pylorus in frogs in Massachusetts. In 1911 Osborn reported it from St. Paul, Minnesota, the worms occurring near the pylorus and the neck of the bladder. Dr. Simon exhibited specimens of the worms and the tumors caused by them, noting that the worms caused a proliferation of the glandular epithelium, the hyperplastic tissue growing through the muscular coats of the stomach. The growth is not necessarily malignant, but is similar to the hyperplasia in bilharziasis, which sometimes becomes malignant. Fluke eggs are found in the cyst cavity and its walls. The lesions show a high local eosinophilia.

In comment Dr. Hall noted that there was apparently some possibility that a solution of the cancer problem might be reached through the field of parasitology, as the dependable production of cancer following infection of rats and mice with *Gongylonema neoplasticum* furnished very valuable material for an experimental study of this disease. It has also been reported recently that malignant tumors can be experimentally developed in 50 percent of experiment mice by infecting them with *Cysticercus fasciolaris*, though later investigations have not confirmed this report. The association of various parasites with the occurrence of neoplasms was also noted in schistosomiasis, mite infestations, etc. Dr. Ransom noted that de Jong found neoplastic growths caused by parasites retrograde with the death of the parasites and no malignant growths develop, but that under certain conditions the persistence of the parasite may lead to the development of serious but non-cancerous growths. Dr. Simon reported that the flukes in frogs may at times occur in the mesentery and Dr. Cort reported finding them in the region of the rectum and bladder.

Dr. Taliaferro presented a note by Taliaferro and Becker on the structure of the iodine cysts. These were first reported from human feces and were subsequently found to be widely distributed. They have been reported twice from pigs, the cysts in these cases showing no recognizable differences from those in human cases. At present the consensus of opinion is that they are encysted amebae. They are small, round forms 7 to 12 $\mu$  in diameter, with a glycogen center taking the iodine color. Kofoed has regarded them as forms of

*Endolimax nana* and certain staining reactions suggest this relationship. However, a critical examination of these forms does not bear out this conclusion and it appears that the iodine cysts are not *Endolimax*. The cysts of *Amoeba fragilis* have not yet been found, and a case of infection with this protozoan in Baltimore is reported by the authors.

Dr. Cort presented a note on sources of soil infestation with hookworm larvae. (See Investigations on Control of Hookworm Disease, etc.)

Dr. Hall presented the following note by Dr. Cobb:

*Notes on the Adhesion-Tubes of Draconema cephalatum*

The following observations were made on specimens of *Draconema cephalatum* from Hudson's Bay, collected by Mr. Fritz Johansen and forming a part of the Canadian Arctic Expedition material. The specimens were fixed with alcohol-formalin and stained with acid carmine.

The adhesion tubes, each connecting at its base with a duct leading to a distinct gland, are arranged in a long ventral group opposite to and a little in front of the major sole. The anterior pair of glands, which is sometimes slightly separated from the general mass of the glands, is composed of two glands having three nuclei each. The cells of each gland, as well as their nuclei, diminish in size from back to front, the anterior cell having less than one-eighth the volume of the posterior. Posteriorly, each gland diminishes suddenly in diameter to form a duct, which leads backward and outward to the seta and which is from one and one-half to two times as long as the gland. Where the duct enters the somewhat swollen base of the adhesion tube, there is a small duplex enlargement or ampulla. These glands are so closely packed together that it is difficult to distinguish the exact number of groups, but it is evident that throughout the series, the glands are arranged in groups side by side, apparently mostly in pairs or quartets.

Dr. Hall presented a note by Hall and J. E. Shillinger, entitled "Miscellaneous Tests of Carbon Tetrachloride as an Anthelmintic." The paper reviewed the published literature dealing with tests on dogs, horses, and monkeys, and the one report of the results of administering an experimental dose of the drug to man. Additional tests on dogs, foxes, swine and sheep were reported. The drug seems to be specifically valuable for the removal of the blood-sucking strongyles, removing 100 percent of the worms present in the case of the hookworms of dogs and, apparently, of foxes, of stomach worms and hookworms of sheep, and of *Strongylus* of horses. It was surprisingly effective in removing the small trichostrongyles of sheep, removing 82 percent of 800 worms present in 3 sheep. It is quite effective against ascarids in dogs, swine and horses, though rather large doses are required in the case of swine. When used in large doses it is rather effective against whipworms. So far the drug has been found to have an unusually high safety factor. Dogs show no bad effects after doses of 5 c.c. per kilo of live weight, and as the therapeutic dose is 0.3 c.c. per kilo, the safety factor appears to be at least 17 and is probably higher. Foxes have survived doses of 2.7 c.c. per kilo, though these animals are extremely susceptible to poisoning by anthelmintics. Monkeys survived doses of 6 c.c. per kilo. On the basis of the experimental findings on animals the drug is now being tested on man by physicians as a treatment for hookworm infestation.

The paper was discussed by Drs. Simon, Shields, Cort and Curtice.

The fifty-sixth meeting was held December 10, 1921.

Dr. Hadwen spoke on parasites he found in reindeer and other animals in northern Alaska during 1920-1921. Some herds were very lightly infested. Among the parasites found were screw worms (*Phormia* sp.), warbles (*Oedemagena tarandi*), grubs in the head (*Cephenomyia nasalis*) and various species of Sarcosporidia. One species of Sarcosporidia was found in the heart and another in brownish cysts in the legs. A third species is apparently a *Balbiana* or a closely related form. A sarcosporidian with very large cysts up to 2.2 cm. long was found in seals. It was observed that reindeer harbor

the largest number of warbles during the first year of life; approximately half as many were present during the second year, and very few thereafter. The injection and instillation of juices from larvae of *Cephenomyia* and *Oedemagena* produced little evidence of anaphylaxis. If these forms produced serious anaphylaxis it would be a matter of great importance, as some animals harbor as many as 1,000 warbles. Actually the anaphylaxis is much less marked than that produced by *Hypoderma*. The adult *Oedemagena* causes the reindeer to "mill," or travel in a circle, but does not cause as much fright as *Hypoderma*. On the other hand, *Cephenomyia* does frighten reindeer. Upon the approach of one of these flies, as it poises in front of the animal's nostrils, the animal stands as if petrified, with lowered head, staring eyes and outspread legs. The reindeer starts violently when struck by the fly. Later there is some nasal irritation with discharges from the nostrils. This parasite is difficult to control. The control of *Oedemagena* is also a difficult problem. Moving the herds after the larvae have left the reindeer is a useful control measure, and building sheds for shelter may be of value.

*Cysticercus krabbei* was not found to be as commonly present, or as numerous as was expected. *Cyst. tenuicollis* was common and hydatids were occasionally found in the lungs, never in the liver. Species of *Moniezia* were very common, especially in fawns, and the observations made indicate that these worms grow very rapidly. Species of *Nematodirus* are apparently very common and serious parasites, and in one case were found associated with a marked anemia. Two species of this genus, as yet undescribed, occur in Alaskan reindeer. A species of *Dictyocaulus*, a large form, is common in the bronchi.

The paper was discussed by Dr. Stiles and Dr. Bartsch, the latter raising the question as to the origin of the existing parasites of Alaskan reindeer. Dr. Hadwen thought that they were imported with the reindeer. A herd on one of the islands of Alaska was found practically free from parasites, probably as a result of the reindeer originally brought to this island having been without parasites or only slightly infested. He noted that in one case fawns had been put on an island 14 miles from the coast after all visible grubs of *Oedemagena* had been removed, but as many eggs were found on these animals the next fall it is probable that a sufficient number of grubs escaped to keep up the infestation. The fly is very resistant, apparently, to water and frost. The eggs of this fly are mostly laid on the line of contact of the animal with the soil, especially about the hocks and knees. The flies back up to oviposit and may lay as many as 25 eggs on one hair. The eggs hatch spontaneously without extraneous stimulation. Dr. Hadwen stated that caribou skins free from warble holes were a great rarity.

Dr. Curtice noted that *Nematodirus* in sheep is a serious pest, causing distinct injury and evident losses when present in large numbers where there were very few stomach worms present. Dr. Ransom stated that the species of *Nematodirus* found in reindeer are large, one of them being very large.

Dr. Hall noted that *Oestrus ovis* of sheep appear to be controlled in a rather satisfactory manner by the use of pine tar smeared on the nose. Dr. Hadwen stated that no control measure of this sort had been attempted in the case of *Cephenomyia*; the grub was often covered with blood when sneezed out.

Dr. Bartsch discussed the native fauna of Alaska, noting the various theories as to its origin. In some areas there was no glaciation during the Glacial Epoch, the fauna persisting at a time when the fauna south of these areas was wiped out or driven back. After the retreat of the ice sheet the Southern fauna worked north to meet the surviving Northern fauna.

Dr. Hall presented the following note:

*A Case of Apparently Spurious Parasitism in Man*

Under date of July 29, 1921, Dr. St. J. B. Graham of Atlanta, Georgia, sent some specimens to Dr. L. O. Howard, U. S. Bureau of Entomology, with the following letter: "I send you today two or three larvae passed about two



years ago by a mountaineer from the bladder with the urine. He passed many of these and they flipped about on the ground when he made or passed urine in the open. A physician brought out some with a catheter; passed them while snow was on the ground, winter and summer. . . . They do not answer to ordinary larvae of urinary miasis, *Fannia scalaris* or *Fannia canicularis*. All my dealings with this case were by mail. . . . The patient was extremely nervous, but has ceased to pass larvae some months ago." The specimens in question are small oligochaetes, evidently free-living forms which can scarcely be regarded as parasitic. It is hardly conceivable that they could occur in the urinary bladder of man unless they were introduced there. It would appear that the patient's mentality and habits should be investigated. As the patient was said to be extremely nervous, it is probable that some mental derangement is associated with this case.

In comment Dr. Ransom noted that similar cases often come to the attention of the government laboratories and that they are almost always poorly defined and involve probable errors of observation or of interpretation. Dr. Stiles exhibited a specimen of *Paragordius varius* from Indianapolis, Indiana, with the history of having been passed from the urinary bladder of man.

Dr. Stiles also presented a note on the nomenclature of Amoeba. The original name used for animals belonging in what might be regarded as amebae, was Chaos. Chaos appears to be undefinable and it seems advisable to suppress it. The nomenclature of the pathogenic and non-pathogenic amebae of man is very difficult and unsatisfactory and very much complicated. However, a fair case may be made out for retaining *Endamoeba coli* as the name of the non-pathogenic form and *Endamoeba histolytica* as the name of the pathogenic form. At present it appears probable that too many genera are being made in this group. It would seem advisable to retain *Endamoeba* for the foregoing parasitic forms and to put other forms in subgenera until they are established on a sounder basis.

Dr. Ransom presented a note illustrated with lantern slides, dealing largely with plans for making the results of investigations of economic importance available for practical use in the control and eradication of parasites, with reference particularly to ascaris in swine. The paper was discussed by Dr. Stiles and Dr. Curtice, who pointed out the need for greater practical utilization of our present knowledge.

The fifty-seventh meeting was held on January 21, 1922. Attention was called to the fact that the Royal College of Veterinary Surgeons of England had awarded the Steel Memorial Medal to Dr. Albert Hassall.

The following American corresponding members were elected: Ackert, Barber, Boeck, A. C. Chandler, van Cleave, Graybill, May, Riley, Schwartz, Scott, Stunkard, Wigdor and Wolbach.

Dr. Stiles reported that the International Committee on Zoological Nomenclature had accepted for the official List of Generic Names the names proposed by the society, and he raised the question as to the advisability of submitting additional names. A committee was appointed to draw up such a list of names and report. Dr. Stiles suggested that there should be a Code of Ethics for zoologists, and he called attention to the harsh and caustic criticisms sometimes made in publications in regard to other zoologists.

Dr. Stiles discussed the possibility that the recent war had resulted in a spread of amebiasis in the United States, presenting in this connection some statistical findings by Dr. Boeck. Kofoid has found amebiasis in over 12 percent of 2,300 returned soldiers in a New York debarkation hospital, and in 67 percent of veterans who are students at the University of California. According to Kofoid a single stool may contain 50,000,000 cysts; he estimates that 3,000,000 overseas soldiers have been exposed and that probably over 300,000 are carrying amebiasis back into this country. On these grounds he urges that the United States examine and treat returned soldiers.

According to Dr. Stiles, it costs about \$1.20 to make a fecal examination for parasites, or \$0.60 to examine for amebae alone. The costs for microscopic examination, collection and hospital expenses would total about \$32,400,000.

Before incurring any such expense, one must be very certain of its necessity. To secure information on the prevalence of amebiasis in this country, circulars were sent to 607 hospitals and 115 medical schools in regard to clinical cases of amebiasis found by them. In the 532 replies from 44 states, 24, or 4.4 percent, were affirmative for cases seen. These affirmative were indefinite and usually referred to one or a few cases. The Hygienic Laboratory examined 13,047 samples from 8,029 persons, chiefly in government hospitals. *Endamoeba histolytica* was found in 4.1 percent, most cases being carriers without clinical symptoms. Doubtless more would have been found infected if 6 examinations per capita had been made. Groups examined included in addition to the government hospital group, Ellis Island immigrants, persons at the Reform School and the Industrial School at Washington, D. C., persons in the military service at home and abroad, and various civilians. The Industrial School had amebiasis in 12 percent of the persons examined, though no clinical cases have ever been observed at the school. In undertaking a large project, such as the treatment of the returned soldiers, one must take into consideration principle, policy and price. The studies of amebiasis in the groups examined in this country show that the returned soldier is not a menace and that the war has not materially changed the situation in the United States as regards the prevalence of amebiasis. Furthermore, there is no law under which the returned soldiers could be ordered examined, any more than soldiers not going abroad or civilians could be made to submit to examination. So far as the urban population is concerned, there is little likelihood of the cysts of *E. histolytica* which are carried away in sewage being returned to man as infective agents. Where privies are used, there is some danger that part of the cysts would be carried back to man by insects, though most of the cysts die. Where there is no sanitation the danger is greater. It appears then that clinical control is not advisable, but that sanitary control is the important thing. If the federal government is to spend \$30,000,000 on control, it should be spent on sanitation; a smaller amount spent in this way would do more good than a larger amount spent on clinical control. A patient with clinical symptoms will take medicine; as a rule a carrier will not. So far as determined, from 5 to 7 percent are carriers of amebae, and this itself is reason for sanitation, but not for clinical treatment. The war has shown that we have exaggerated the importance of protozoan infections of the intestine. The claim has been made that fecal examinations for protozoan parasites should be made in every hospital for every patient. This would cost at least \$0.50 and a private laboratory would charge \$5.00 or more. Stiles has recommended that such examinations be made as routine for all patients in or from the South, and otherwise only in cases showing anemia, intestinal disturbances or amenorrhea of unexplained origin. For other cases it is not practical and is too costly.

Dr. Stiles noted that amebae have been regarded as water-borne but in his opinion foodhandlers and the common house-fly are largely responsible for the distribution of the cysts. Among the patients examined at St. Elizabeth's Hospital for the Insane at Washington, D. C., it was found that protozoan parasitism tends to increase with length of residence, as has been found to be the case elsewhere. It appears not unlikely that carriers of amebae employed as cooks, waiters, etc., are responsible for this. At one college, 67 percent of certain men examined (Kofoid) were found to be infected; all of these men served in the same unit in France. A reasonable explanation is that some carrier might be responsible for this infection.

Dr. Butler noted that in 1912 a high percentage of men from one ship at Manila were found infected with amebae, presumably as a result of the concentration of persons in narrow limits in the presence of a carrier. Dr. Stiles stated that extensive studies of amebiasis had been made also in India and in England. He also stated that *Councilmania lafleuri* had not been recognized in the present survey, and if it was present and seen it would have been regarded as *E. coli* and included in the figures for that species. Dr. Butler noted that the Navy might bring in many parasites, as ships touch at foreign ports, infected persons without clinical symptoms serving as carriers.

Dr. Ransom presented the following note:

*Observations on the Toxic Effects of Ascaris Fluids*

Repeated cutaneous tests on a human subject (B.H.R.) with fresh fluid from the body cavity of the swine ascarids (pulverized after thorough drying) gave definite and prompt local reactions, a stinging sensation, itching, formation of a wheal, and reddening of the surrounding skin, with swelling. These tests were made by applying a drop of the fluid or particles of the powder to a scratch made in the skin through the epidermis without drawing blood but exposing the cutis. The tests were made at varying intervals during a period of several months between December, 1917, and May, 1918. The first test was made 3 days after a conjunctival reaction (itching, reddening and swelling) of which the accidental introduction of *Ascaris* fluid into the eye was suspected of being the cause, a suspicion confirmed by the prompt appearance of a local reaction following a cutaneous test. A second test later in the same day was also followed by a pronounced local reaction. A third test made with powdered *Ascaris* 5 days after the second test gave a pronounced local reaction and 10 hours after the first appearance of the reaction which came on within 5 minutes, the skin at the site of the reaction was still reddened and tensely swollen over an area 3 cm. in diameter, with a red streak 25 cm. or more long, extending up the arm from the swollen area. Another test in April, 1918, using boiled *Ascaris* fluid gave a similar reaction. Eighteen hours later a blood smear was made. A differential count of the leucocytes showed 26 percent of eosinophiles. A total leucocyte count was not made. Beginning 20 days later differential counts of the leucocytes (no total counts made) on several days gave percentages of eosinophiles varying from 1.4 to 3 percent, neutrophiles varying from 53 to 65 percent, and mononuclears (large, small, and transitional) varying from 33 to 44 percent. On May 3 and May 6 tests were made with dried *Ascaris* powder, giving positive but slight reactions. There were no marked changes in the differential count of the leucocytes following these tests (total counts not made). On May 7 at 11 o'clock, a drop of fresh *Ascaris* fluid accidentally fell on a very small, slight, recent abrasion of the skin of the wrist; this was followed in less than 5 minutes by the usual appearance of a pronounced local reaction, and these very soon by general urticaria, which at 11:15 was well established over the entire body. The face and eyes were swollen. The heart was very rapid, beating at the rate of at least 150 per minute. There was a sensation of warmth and breathlessness. At 11:30 the symptoms were beginning to subside, the heart was slower. An hour after the beginning of the reaction the urticarial wheals were still present; after another half hour the wheals had become red, the face and eyes were less swollen. At 1 o'clock the arms and body were spotted with red, the eyes only slightly swollen. On the arm surrounding the abrasion the skin was reddened and swollen over an area several inches broad, with a red streak extending up to the shoulder from this area. At 4 o'clock, 5 hours after the beginning of the reaction, the red spots on the body had become very faint, the face and eyes were slightly swollen, the arm in the region of the abrasion much swollen, but only faintly red. The next day the affected region of the arm was still somewhat swollen, the face slightly swollen. On May 9, two days after the occurrence of the reaction, the arm continued slightly swollen, but on May 10 the swelling had entirely disappeared. A blood smear was made two and a half hours after the beginning of the reaction. A differential count of the leucocytes (no total count made) showed 82.5 percent neutrophiles, 17 percent mononuclears, and 0.5 percent eosinophiles. Five and a half hours after the beginning of the reaction the neutrophiles were 79.5 percent, mononuclears 20 percent and eosinophiles 0.5 percent (differential count only). In the afternoon of the following day a differential count gave neutrophiles 60 percent, mononuclears 36 percent and eosinophiles 3.5 percent.

These observations confirm observations made by Bastian, Leuckart, Railliet, Linstow, Goldschmidt and others as to the more or less severe effects of the secretions or body fluids of ascarids upon susceptible individuals, and show also

that the absorption of minute quantities of the offending substance may not only cause alarming and distressing symptoms, but also greatly disturb the equilibrium of the blood, as evidenced by the changes seen in the relative percentages of the different varieties of leucocytes. The absence of total leucocyte counts in the present instance, as well as the fact that the various changes in the blood that have been observed by different investigators following the injection of verminous toxins and a variety of foreign proteins into experimental animals have not been well correlated, does not permit broad conclusions to be drawn as to the significance of the results of blood counts in this case. The experiences with *Ascaris* fluid indicate a possible risk in cutaneous tests of susceptibility to foreign proteins, now very commonly made on patients suspected of foreign proteid sensitization. It was also suggested, as may already have been suggested by others, that some of the recorded cases of sudden death in which wandering ascarids have been found in the larynx or trachea may have been caused by edema accompanying a local reaction of the mucous membrane of the larynx to contact with the worms.

In comment on the cases where eosinophiles are rare in the circulation, Dr. Hadwen noted Weinberg and Seguin's findings to the effect that eosinophiles left the circulating blood to mass at the point of injection of parasite fluid. Dr. Butler noted that eosinophilia is commonly present in cases of urticaria, a matter of interest in connection with Dr. Ransom's case where a marked urticarial reaction was accompanied by a low eosinophile count. Dr. Ransom called attention to Hadwen's experiments where *Hypoderma* juices contaminated with bacteria were used; the eosinophiles engulfed the bacteria which had been opsonized with *Hypoderma* fluids. Dr. Stiles reported that at one time Railliet had suffered from the effects of ascaris toxins and had discontinued wearing the laboratory attire worn during the work on ascarids. Some time afterward this laboratory suit was put on and promptly brought on a recurrence of the original symptoms.

In reply to the question as to whether ascarid infestation was involved in the reaction to ascarid products, Dr. Ransom noted that sheep, not usually infested with ascarids, can be easily killed with small amounts of ascarid fluid injected subcutaneously, whereas guinea-pigs will tolerate large amounts, even when injected intraperitoneally. Dr. Joseph Leidy, Jr., told Dr. Ransom that the removal of ascarids from a child has been followed by the subsidence of a conjunctivitis. Dr. Butler stated that asthma in Guam may be associated with ascariasis. Both conditions are very common and the asthma is often fatal, especially in children. Dr. Cort reported that many cases of asthma in Trinidad recover after successful treatment for hookworm disease. Dr. Ransom noted in this connection that extracts of hookworms or of *Hacmonchus contortus* would kill sheep.

On the question as to why worms in the intestine are less injurious than their products when injected, Dr. Ransom offering the explanation that the intestine is probably a more resistant tissue for the particular attack it sustains. Dr. Hadwen noted that animals commonly lick the site of injection when parasitic material is injected. Dr. Ransom stated that hemolysis by worm products can be demonstrated *in vitro*. When asked if entire ascarids would produce anaphylaxis, as these worms were commonly used as laboratory material, Dr. Ransom stated that the entire worms could give rise to toxic effects and that susceptible students might become affected. Dr. Stiles stated that he has seen such cases in a few students, itching at the nose being a common symptom. Dr. Harper noted in this connection that certain individuals were very sensitive to horse dandruff.

Dr. Hall presented the following note:

*Alaria americana from the Cat*

A specimen of *Alaria* from the small intestine of the cat was sent in to the Zoological Division of the Bureau of Animal Industry by Dr. Wm. A. Riley in December, 1921. Dr. Riley states that two specimens were collected from



a cat at University Farm, St. Paul, Minnesota, but one of the specimens was lost. The remaining specimen was compared with the descriptions of various known species and with the type and paratype specimens of *Alaria americana*, and appears to be an immature specimen of this species described by Hall and Wigdor in 1918 from the small intestine of the dog at Detroit, Michigan. They found this fluke in 4 of 300 dogs, 91 flukes being present in one animal. Dr. Riley's findings indicate that this fluke occurs in the cat as well as in the dog, and in Minnesota as well as in Michigan.

*Oxyuris compar* Leidy, 1856, a Synonym of *Oxyuris ambigua* Rudolphi, 1819

Leidy reported *Oxyuris compar* from the small intestine of the cat. Some later writers include it as a parasite of the dog also, but nothing in the literature warrants this inclusion. The fact that this worm, of which only the female is known, was found in the small intestine, whereas worms of this genus are usually parasites of the large intestine when adult, suggests that it was out of place and in a strange host. This is further indicated by the fact that the worm has only been found once. It seems probable that the cat had eaten some mammalian host normally parasitized by this worm and was examined postmortem while the worms of the ingested host were still present. The description of *O. compar* corresponds rather closely with that of *O. ambigua* of the rabbit, and rabbits are not infrequently eaten by cats. The measurements and proportions correspond very well. Leidy states that the tail of *O. compar* is spirally contorted. This description might well be applied on casual examination of a worm, perhaps distorted or partially digested, with the annulate tail structure of *O. ambigua*. Probably *O. compar* is not a normal parasite of cats, but a rabbit parasite found in a cat which had eaten the intestine of a rabbit.

The fifty-eighth meeting was held on February 18, 1922. The following foreign corresponding members were elected: Bayliss, Ciurea, Fibiger, Joyeux, Katsurada, Seurat and Travassos. The proposition that the society consider a code of ethics was discussed, and a committee to formulate a tentative code was named as follows: Drs. Cobb, Cort, Bartsch, Butler, Stiles and Hall.

Mr. Chapin presented the following *Note on Monostomes*.

Monostomes are not uncommonly found in the body cavity and suborbital cavities of shore birds, such as species of *Totanus*, *Fulica*, etc. These flukes have been very generally referred to the species *Cyclocoelum mutabile*. Kossack, however, recognizes 7 species. An examination of specimens in the Bureau of Animal Industry collection shows certain differences in the egg sizes and in other respects between specimens from *Totanus* and specimens from *Fulica*. There are two lots of specimens from *Fulica americana* available for study. In these specimens the muscular portion of the pharynx is of a diameter equal to or slightly greater than that of the posterior testis. The average length of the eggs of this species was 105.6 $\mu$ . In these characters, the specimen showed a close relationship to the European *C. microstoma* Crep., from *Fulica atra*. Material from *Totanus* sp. collected in Mexico showed a much smaller pharynx and larger eggs, the average length of the latter being 135.4 $\mu$ . These characters more or less parallel the characters of *C. problematicum* Stoss. from *Totanus calidus*. In comment Dr. Ransom noted it as a matter of great interest that these differences, associated with different hosts, should be duplicated in this country and in Europe.

Dr. Cobb exhibited a microscope for the rapid examination of large fields, and also a number of miniature Syracuse watch glasses of various types, some of glass and some of glass and brass. The ordinary Syracuse watch glass is too large in many cases where small specimens are being handled, as it is difficult to find the specimens and the large glasses are wasteful of fluid. It is also much cheaper to grind the base of the small glasses to secure opacity.

Dr. Cobb presented a note on the coconut nema of Panama, with specimens of the nema. With other workers Dr. Cobb has recently investigated conditions

in Panama where this worm constitutes a serious pest. The nema is about 1 mm. long and very slender, and belongs to *Aphelenchus*, a genus which includes many important pests. It infests the roots and from here enters the trunk and ascends to the crown. In about 3 weeks there is a rapid change in the tree, followed by its death. The worms are present in enormous numbers, and cause an oxidation change in the palm, the tissues turning red, whence the name of red ring applied to the disease. This red ring forms a cylinder, 1 to 1.5 inches wide under the bark, showing as a ring in cross section. The disease has spread completely around the Caribbean Sea and is probably very old; the many plantations which have been wiped out several times in this region were probably destroyed by this nema. This is a serious matter, as the coconut is being grown on a large scale for the last 10 or 15 years. Coconut oil is much more widely used than formerly. The disease is most fatal when the trees are 4 to 7 years old. The disease spreads very rapidly.

On looking for a carrier, it was found that a large palm weevil, common wherever coconuts are grown, feeds on the tissues and lays its eggs in the trunk. An examination showed that 50 percent of these beetles carried the nemas. Whenever a palm is cut the weevils assemble rapidly to feed, thereby gaining access to diseased tissue, which has an ethereal odor and seems to be preferred by the weevils. When the hairy mouth parts are thrust into diseased tissue, the nemas are transferred to the hairs and also get onto the hairs of the legs. They have not been found in the intestine of the beetle. The transfer of the nemas is therefore mechanical. The nemas spread best in humid environments. Old palms do not suffer, nor those on the beaches, but the younger ones farther back from the beaches. The palm can tolerate large amounts of salt without injury and thrive in its presence. The tissues of shore trees are toughened by the wind and the nema does not get as good food from them. They thrive best in the more succulent tissues of rapidly growing trees away from the beach.

The nemas were thought to be apogeotropic because they ascended objects in infested soil. But they also wander down, moving either way in the dark, but ascending in light. Apparently they are phototropic. The nemas are not found on the surfaces of the palms and do not climb the trees, but probably start as root parasites. There are numerous tough roots to a palm, but the nemas probably enter between these and ascend the trunk. Dr. Cobb stated that the worms tolerated sea water very well but not heavy brine. The use of salt in kelp at the base of the palm is of value in keeping out the nemas and the beetles. The nemas are found in the husk, which is planted with the remainder of the nut. They also occur in young trees, but the trouble begins when the trees are 4 years old. Perhaps the rapid putting out of roots earlier aids in keeping the nemas down. Dr. Schapiro also noted that some writer had reported "hookworms" from bananas.

By vote the secretary was instructed to record a vote of congratulation to Dr. Hassall on the award of the Steel Memorial Medal.

The fifty-ninth meeting was held on March 18, 1922.

Dr. Cort presented a note on *Fork-tailed Cercariae*. In 1914-1916 he reported on two such cercariae of this type being long known, but not thought of medical interest as their relationships were unknown. Miari and Suzuki and also Leiper later investigated the life history and found that these forms were the cercariae of the schistosomes. At first it was impossible to determine the species of the adult from a study of the larvae, but subsequent work has established the relations of the larvae to the corresponding adults, the excretory system and cephalic glands being structures of great diagnostic value. Indications of sexual dimorphism in the cercariae have also been found.

Dr. Cort also reported that the field work on hookworms, begun last summer in the Island of Trinidad for the International Health Board, would be continued next summer in Porto Rico, and that besides himself Dr. and Mrs. Payne, Dr. W. A. Riley and Messrs. Stoll and Augustine would be members of the party.

Dr. Stiles noted the importance of the importation of flukes by immigrants, not only as a matter of medical interest, but because of its bearing on the welfare of the livestock and fishing industries.

A paper on *The Rat Tapeworm*, *Hymenolepis diminuta*, in *Man*, by Drs. Wm. A. Riley and W. R. Shannon was read in abstract (see this JOURNAL, 8: 109-117, March, 1922).

Dr. Cobb exhibited some holders which he had devised for safety razor blades to make an instrument for cutting sections in laboratory work.

The sixtieth meeting took the form of a dinner on April 1, 1922, and the presentation to Dr. Albert Hassall of the Steel Memorial Medal of the Royal College of Veterinary Surgeons of England. The medal was formally presented on behalf of the college by Dr. Seymour Hadwen.

The sixty-first meeting was held on April 22, 1922. The following officers were elected for the ensuing year: Dr. Hall president, Mr. Chapin recording-secretary, Miss Cram corresponding secretary-treasurer.

A communication from Dr. Goldfarb, with reference to the cooperation of the society with Section N, the section on medical sciences, of the American Association for the Advancement of Science, was read and discussed. It was voted that the society cooperate and that Dr. Cort act as its representative. A committee consisting of Drs. Ransom, Hall and Butler was appointed to consider borderline topics for Section N and naming those to take part in the symposium.

The Code of Ethics proposed at previous meetings was brought up for consideration with suggested amendments. The code as adopted is as follows:

#### CODE OF ETHICS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

1. Adverse discussion of the work of another scientist should be impersonal. Any disagreement with the statements of any other writer should be expressed in terms of disagreement with the statements only and should not be expressed in a manner likely to reflect upon his competence or intelligence.

2. When specimens are examined by two or more persons with a distinct understanding or a definitely implied agreement as to publication, whether jointly or independently, the terms of the understanding or agreement should be observed as rigidly as in any contract.

3. When specimens are examined by two or more persons, as when specimens are referred by collectors to systematists, all persons concerned may retain the right of independent publication in the capacity in which they act.

4. In correspondence or conference, statements of new and unpublished findings are to be regarded as privileged communications, and no persons should publish information thus first obtained unless properly authorized to do so.

5. The general rule covering cooperative activities is that the greatest possible courtesy should be observed by all persons at all times. In general it is not proper for a collector to request a systematist to determine whether an unstudied specimen is new or not and then, if determined as new, to claim the right of description for publication; but where it is desirable that material be compared with certain type specimens, the right of description may be retained by the collector, preferably by definite agreement with the person making the requested comparison with type material.

6. As recommended by the International Zoological Congress, persons noting that homonyms of existing species or genera have been published by a writer, should refer the matter to the author, notifying him of the homonym. It is here considered the duty of the author of the homonym to propose a substitute name immediately and to publish it promptly, at the latest within a year from the receipt of such notification. After one year, or at the suggestion of the author that someone else propose a new name, it is here considered proper for anyone to propose a new name.

7. The transfer of a species from one genus to another, one family to another, etc., is properly within the province of any writer without referring the matter to the author of the names involved; such transfer is a matter of individual opinion and the acceptance or non-acceptance of the combinations arrived at is a matter of consensus of opinion among zoologists.



8. In general, authors should avoid in their publications anything which shall seem to claim as original, ideas, discoveries or illustrations which are not original with them. For many generally or long known facts it is often obviously unnecessary or impossible to cite authority; it is, however, possible to avoid writing in a manner which seems to claim credit for subject matter or wording for which the credit belongs to another.

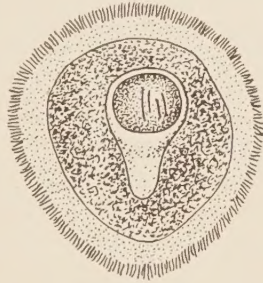
9. An official or superior should not take advantage of his position in connection with any publication resulting from work performed by his students or assistants, even when they are working under his immediate direction. Conversely, students, assistants and others should respect their obligations to their official superiors and colleagues.

10. When called into professional consultation by a practicing physician, an active member of this society should govern himself by the Principles of Medical Ethics adopted and published by the American Medical Association.

11. When called into professional consultation by a practicing veterinarian, an active member of this society should govern himself by the Code of Ethics adopted and published by the American Veterinary Medical Association.

12. In case of war, epidemic, or epizootic, in which technical zoological knowledge is of practical importance to the public authorities, zoologists should consider themselves at the service of such authorities, whether federal, state, or local.

13. A zoologist should acknowledge the right of an editor to edit copy in harmony with the established editorial rules of his journal, in so far as



Text Fig. A

language and typography are concerned. In matters of fact and nomenclature, namely Latin systematic names, the author is considered to have the right of final decision.

The undersigned active members of the Helminthological Society of Washington, recognizing that a formal professional Code of Ethics will have a tendency to harmonize views occasionally at variance, agree to adopt the foregoing principles. [Signed by all active members.]

The recording secretary presented the following notes by Dr. Hadwen:

*The Egg of Schizotaenia americana (Stiles) a Porcupine Tapeworm*

In examining the eggs of tapeworms collected from *Erethizon epixanthus* in Alaska it was noticed that the outside shell of the egg was covered with minute spines, irregularly scattered over its entire surface. While a complete study of the tapeworm has not been made it seems identical with *Schizotaenia americana*. Material from *Erethizon dorsatus* in the collection of the zoological division of the Bureau of Animal Industry was also examined and the egg found to be exactly similar. Douthitt (1915) gives the measurements of the ova as 12 to 15 $\mu$  in diameter. He also states that there is no pyriform apparatus attached to the pyriform body and this fact has been confirmed in the examination of the Alaskan material (Text Fig. A; see above).



*Nematodirus tarandi*, a New Species of Nematode from the Reindeer

*Specific Diagnosis.*—*Nematodirus*: Male averages 12 mm. in length by  $75\mu$  in width. The smallest specimen measured was 11.5 mm. long and the largest 13 mm. long. Spicules 2.72 mm. long and provided with shoe-shaped terminal piece. Esophagus about  $300\mu$  long. Female averages 16 mm. in length by  $175\mu$  in width. The smallest specimen measured was 13 mm. long and the largest 19 mm. long. The vulva is slightly anterior to a point two-thirds of the body length from the head end. The anus is about  $80\mu$  from the tail end. Eggs 75 to  $100\mu$  long by 50 to  $75\mu$  wide.

*Host.*—Reindeer, *Rangifer tarandus*.

*Location.*—Small intestine.

*Distribution.*—Alaska.

*Type Specimens.*—United States National Museum Collections No. 24611 (type and allotype) and No. 24960 (paratypes). Collected from reindeer at Egavik, Alaska, August 28, 1920, by Seymour Hadwen.

The recording secretary presented the following notes by Dr. Wm. A. Riley:

*Dirofilaria immitis* in the Heart of a Cat

I recently received from Dr. D. W. Davis, of the College of William and Mary, at Williamsburg, Va., two specimens of round worms from the heart of a cat. One of the specimens was incomplete. The other measured about 20 cm. in length and when found extended from the right auricle for about 3.5 cm. up into the precava. The other specimen was in the right ventricle, greatly distending that cavity. The worms proved to be *Dirofilaria immitis*, both being females. As far as I have noted, this well known parasite of dogs has not been reported for the cat. Horst (1889a) reports it from the right ventricle of the heart of the jaguar, *Felis onca*, and Kitt (1915) found specimens in the heart of the Sumatran tiger, *Felis sondiacus*. In this connection I might mention that I have seen two unreported cases in dogs in New York and that Dr. Johns of Tulane informed me that "half the dogs of New Orleans were infested." While this statement may not have been intended literally, it is at least evident that *Dirofilaria immitis* is not as rare in this country as has been supposed.

*Additional Cases of Hymenolepis diminuta* in Man. (Published in this JOURNAL, March, 1922.)

Dr. Ransom, in comment, discussed the relative frequency of *Taenia saginata*, *H. nana* and *H. diminuta* in the United States, noting that *T. solium* was very rare. *Cysticercus cellulosae* is correspondingly rare in swine in this country. In an examination of one million swine during the course of about one week, only 28 cases of infestation with this parasite were found. In some sections of the country, where there are a large number of Mexicans in the population, such as the region about Fort Worth, Texas, a larger number of cases are found and swine from these regions are given especial attention in meat inspection. Infestation is usually gross, to an extent not true for *Cyst. bovis*, and can be detected readily on routine inspection. This heavier infestation is due to the fact that the segments of *T. solium* commonly pass in chains whereas those of *T. saginata* pass as single segments, and to the fact that swine are commonly coprophagous, whereas cattle, as a rule, are not. He also noted the increasingly large number of cases of *Diphylobothrium latum* originating in this country.

Dr. Cort commented on the large number of cases of *H. nana* in the South, some localities showing 1 to 7 percent infestations. Dr. Shillinger reported that in one year's experience in meat inspection he had only found 1 case of infestation with *Cyst. cellulosae*, this case showing a very heavy infestation. Dr. Ransom discussed the intermediate hosts and life history of *H. diminuta* and noted that its removal was apparently not difficult. He also noted that the rat and human forms of *H. nana* should be regarded as identical or at least as forms belonging to the same species and with only biological differences in the way of host adaptation.

Miss Cram presented the following notes:

*New Records of Horse Strongyles from the United States*

The following horse strongyles not heretofore reported from the United States have been found post mortem at Bethesda, Md.:

*Oesophagodontus robustus*, *Cylicostomum goldi*, *C. auriculatum* and *C. euproctus*. The following species, also from horses at Bethesda, Md., have been reported in England by Yorke and Macfie from horses which were purchased in the United States, but apparently have not been heretofore reported in this country:

*Triodontophorus tenuicollis*, *T. brevicauda*, *Gyalcephalus equi*, *Cylicostomum coronatum*, *C. pseudo-catinatum*, *C. nassatum* var. *parvum*, *C. longibursatum* and *C. minutum*.

*Crassisoma urosubulatum in the United States*

Worms from the small intestine of a pig which were sent in March, 1922, by Dr. Raffensperger from Chicago, proved to be *Crassisoma urosubulatum*. This is the third infestation with this parasite known in this country; Dr. Ransom reported it first at the November, 1920, meeting, noting two cases. Dr. Raffensperger writes that he found the worms present in large numbers in two pigs from Alabama. This species may therefore prove to be more common than has been supposed.

Dr. Cort presented a note on the escape of cercariae from snail hosts (see this JOURNAL, 8:177, June, 1922).

In comment, Dr. Ransom noted that schistosome cercariae live but a short time after their escape from their snail hosts and that Leiper has suggested the storage of water for a short time to allow such cercariae to die; they apparently live only 24 to 48 hours.

MAURICE C. HALL, *Recording Secretary*.



## NOTES

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An expedition from the Department of Medical Zoology of the School of Hygiene and Public Health, Johns Hopkins University, during the past summer carried on investigations in Porto Rico on hookworm disease. The expenses of the work were paid by the International Health Board of the Rockefeller Foundation. Those who took part in the researches were Dr. Florence K. Bayne, Mr. N. R. Stoll, Mr. D. L. Augustine and Dr. W. W. Cort of Johns Hopkins University, Dr. W. A. Riley of the University of Minnesota and Drs. G. C. Payne and R. B. Hill of the International Health Board. The headquarters of the expedition were in Utuado, where a small hospital was furnished by the Department of Health of Porto Rico for living quarters and laboratory. Two kinds of work were undertaken, viz., field studies on the etiology of hookworm disease and laboratory studies on the development and activities of hookworm larvae.

The field investigations consisted of epidemiologic studies of four areas, by the methods used the previous summer in Trinidad. It was found, as reported by earlier workers, that the degree of infestation with hookworm was much greater in the mountains of Porto Rico than on the coastal plains and that the conditions on the coffee estates were particularly favorable to the spread of hookworm disease. As in Trinidad the intense areas of soil contamination which develop near the houses of the people where the soil is constantly polluted, proved to be important centers of human infestation.

The laboratory researches confirmed the results of the Trinidad Expedition, by showing that under the conditions in Porto Rico, the infective hookworm larvae died out rapidly from the soil, and did not migrate actively far from the place of development. The washing of the hillsides by heavy rains, however, may distribute them over considerable areas. The mixture of feces with sandy soil or humus proved a very favorable medium but development was inhibited in clay soils. All the various phases of the investigation brought out the difficulty which hookworm disease has in spreading from host to host except under gross conditions of soil pollution and in a favorable environment. The results of the summer's work will be published in a series of articles in the *American Journal of Hygiene*.

France is preparing to celebrate in appropriate fashion the approaching centenary of the birth of Pasteur. The celebration will be centered around the University at Strasbourg where Pasteur made his first discoveries. A monument in his honor is to be erected at the University as a result of an international subscription. Furthermore, a Scientific and Industrial Exposition of Hygiene is planned to demonstrate the revolution which his discoveries have brought about in the fields of medicine, hygiene, industry, and agriculture. There is also announced a series of congresses on tuberculosis, cancer, syphilis, etc. These will be held during the period of the Exposition, which is to cover June to October, 1923.